STUDY ON THE PREVALENCE OF HUMAN PAPILLOMA VIRUS INFECTION IN HIGH RISK GROUPS IN A TERTIARY CARE HOSPITAL

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APRIL 2016

CERTIFICATE

This is to certify that this dissertation entitled "**STUDY ON THE PREVALENCE OF HUMAN PAPILLOMA VIRUS INFECTION IN HIGH RISK GROUPS IN A TERTIARY CARE HOSPITAL"** is the bonafide original work done by **Dr. D. KALPANARAJ,** MD Post graduate in Microbiology (2013-2016), under my overall supervision and guidance in the department of Microbiology, Stanley Medical College, Chennai, in partial fulfillment of the regulations of The Tamil Nadu Dr. M.G.R. Medical University for the award of **M.D Degree in Microbiology (Branch IV).**

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DECLARATION

I solemnly declare that this dissertation "**STUDY ON THE PREVALENCE OF HUMAN PAPILLOMA VIRUS INFECTION IN HIGH RISK GROUPS IN A TERTIARY CARE HOSPITAL"** is the bonafide work done by me during my post graduate course in MD Microbiology (2013-2016) at the Department of Microbiology, Govt. Stanley Medical College and Hospital, Chennai, under the guidance and supervision of **Prof. Dr. R. SELVI, M.D.,** Professor of Microbiology, Govt. Stanley Medical College, Chennai, 600 001. This dissertation is submitted to **The Tamil Nadu Dr. M.G.R. Medical University**, Chennai in partial fulfillment of the University regulations for the award of degree of **M.D. Branch IV Microbiology** examinations to be held in April 2016.

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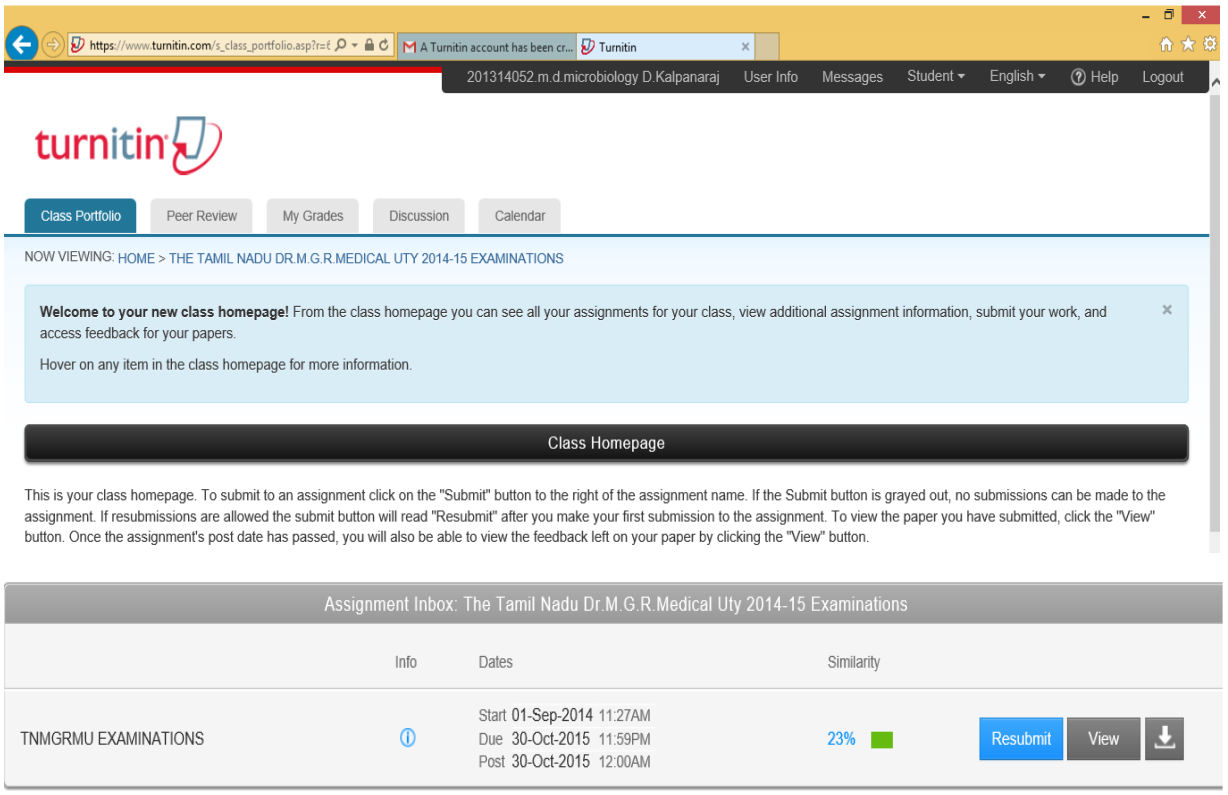
PLAIGARISM



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INTRODUCTION

Sexually Transmitted Infections cause significant morbidity and mortality. Genital Human papilloma virus infection is the most common sexually transmitted infection in the world with an estimated worldwide prevalence of 9 to 13% and approximately 6 million people being infected each year.

AIMS AND OBJECTIVES

The aim of this study is to Determine the prevalence of human papilloma virus infection found in high risk subjects like Female sex workers, Men having sex with men practicing commercial sex, Promiscuous men, Promiscuous women and transgenders practicing commercial sex attending STD clinic in our hospital and to identify concomitant sexually transmitted infections in the study group and emphasize on the need of vaccination against HPV in these high risk groups.

MATERIALS AND METHODS

200 high risk subjects attending STD Clinic our hospital were included in the study. HPV-PCR for HPV 16 and 18 and presence of other STIs were studied and results were observed.

RESULTS

Out of 200 subjects in this prospective study, Promiscuous men and Female sex workers form the majority. The common age group was 31- 40 years. Most of the subjects were married (71%) except MSM and Transgender population. The mode of sex among the study group was heterosexual (81.5%). Among the men having sex

with men, the common type of sex was oro ano receptive (26.6%) kothis, followed by ano receptive (20%). The last sexual contact was protected in 52.5% of the subjects. 62.5% of the subjects had only primary level of education. Concomitant sexually transmitted illnesses were present in 25% (50 out of 200) subjects. Candidiasis was found in13, Bacterial vaginosis in 12, Syphilitic ulcer in 10, Gonorrhoea in 5, Genital warts in 5 and Trichomoniasis in 3 and Herpetic ulcer in 2 of the 50 subjects positive for other STIs. In serological evaluation, HIV was reactive in 14, RPR in 20 and TPHA in 15 subjects. HPV 16 and 18 was positive in 8 subjects by PCR. HPV 16 was positive in 5, HPV 18 in 2 and HPV 16 and 18 in 1 subject. Coinfection in HPV positive subjects with other STIs was present in 5 subjects (Bacterial vaginosis in 2, Candidiasis in2 and Trichomoniasis in 1 out of the 8 HPV positives). Coinfection of HPV 16 and 18 was observed in 1 subject in1, HPV 16 and HIV in 1 and HIV and syphilis in 4 subjects. Blood transfusion and IV drug abuse was observed in 2 and 4 subjects respectively. No HPV positives were found in this group.

CONCLUSION

The HPV 16 & 18 PCR analysis of 200 anogenital and pharyngeal swabs detected 8 HPV positives which included 5 HPV16, 2 HPV18 and 1 HPV 16 & 18. Candidiasis was found in 13(26%), Bacterial vaginosis in 12 (24%), Syphilitic ulcer in 10 (20%), Gonorrhoea in 5 (10%), Genital warts in 5(10%) and Trichomoniasis in 3 (6%), Herpetic ulcer in 2 (4%) of the 50 positive for other STIs. High risk HPV 16 and 18 serotypes are associated with anogenital and head and neck cancers. HPV vaccine induces a protective host immune response which is stronger and long lasting and includes partial cross protection against non vaccine related serotypes. Education

about safer sexual practices, regular screening for Sexually transmitted infections and prophylactic HPV vaccination in this high risk groups will protect the individuals and also prevent the transmission of infection to the general population.

KEYWORDS

FSW – Female sex worker MSM – Male having sex with male, TG – Trans Gender, HPV – Human Papilloma Virus, PCR – Polymerase Chain Reaction.

INTRODUCTION

Sexually Transmitted Infections cause significant morbidity and mortality. Genital Human papilloma virus infection is the most common sexually transmitted infection in the world with an estimated worldwide prevalence of 9 to 13% and approximately 6 million people being infected each year. Human papilloma virus infections are mostly acquired during adolescence or in early adulthood[1]. HPV infections are either asymptomatic and do not not cause clinical disease or may persist clinically as anogenital warts and may progress to precancerous lesions and cancers of the cervix, vulva, vagina, penis, anus and oropharynx. HPV, a non enveloped DNA virus is a member of Papillomaviridae family of viruses. More than 100 HPV genotypes are known of which approximately 40 types infect the anogenital region and around 13 are considered high risk associated with malignancy. Low risk HPV types 6 and 11 cause 90% of anogenital warts. The high risk type 16&18 are known to cause about 70% of invasive cervical cancers and 70% of anogenital cancers in women and 70% of anal cancers in men. HPV types 6&11 are responsible for 90% of genital warts and 90% of recurrent respiratory papillomatosis in both women and men. HPV types 16&18 are also responsible for 40% of vulvar, vaginal and penile cancers and 12% of anal and pharyngeal cancers.

Approximately 250 million women worldwide are HPV DNA carriers. Female sex workers have overall high prevalence of HPV infections of high

risk type[2]. Studies have indicated that having multiple sex partners may lead to higher HPV transmission. Female sex workers[FSW] are thought to be at higher risk of cervical cancer because of high HPV exposure. In addition HPV can be transmitted from female sex workers to the general population through clients thereby increasing the prevalence of the virus. The most highly prevalent HPV types among FSW are HPV 16 [38.9%], HPV 52 [32.7%], HPV 31 [28.4%], HPV 58 [26%], HPV 51 [25%], HPV 33 [25%] and HPV 6 [11.5%] . Infection with multiple and high risk HPV types are common among Female Sex Workers. The overall prevalence is 48.9%, prevalence of high risk types is 43% and low risk type is 24.6%[3]. Infection rate is higher among younger women involved in sexual work for less than one year. HPV prevalence is declining among sex workers with increasing age due to immune response acquired overtime despite continuing high sexual activity[4].

The median overall prevalence of HPV among HIV positive FSW is 73.3% and the most highly prevalent HPV types among HIV positive FSW are HPV 16 [54.2%] and HPV 18 [21.4%][2]. Knowledge of the prevalent HPV types in FSW's may lead to improved prevention measures and assist in understanding vaccination in high risk groups.

The prevalence of Human papilloma virus infection is high among sexually active MSM [male having sex with male] with the anal canal being the most common site of infection. Although many HPV infection in men have been shown to be transient in nature a small percentage persist and can

progress to genital warts, preneoplastic and malignant lesions of the anus, penis and oropharynx and recurrent respiratory papillomatosis[5]. Certain at risk groups such as MSM particularly those with HIV infection are disproportionately affected. The prevalence of HPV is high among transgenders. The high HPV prevalence, the coinfection with multiple genotypes and the high frequency of high risk genotypes detected, together with a extreme situation of marginalization ,discrimination and stigmatization make this population to be of extreme vulnerability[6]. Human papilloma virus is one of the most common sexually transmitted infection worldwide and it causes morbidity and mortality in both men and women via cervical cancer, penile cancer, anal cancer, oropharyngeal cancer and genital warts. While much of the emphasis in the literature has focused on women and link between HPV and cervical cancer, evidence is mounting regarding the high prevalence of HPV infection in males particularly in anogenital sites especially in men who have sex with men [MSM].

HPV is linked to oral, anal and penile neoplasms and also has been associated with acquistion of HIV in MSM. Unfortunately there is little public awareness about the HPV infection and perhaps less so in high risk groups.[FSW,MSM,TG].These high risk groups should be appropriately informed about the social and psychological implications of HPV including its negative impact on quality of life ,mental well being and sexual practices[7].

The purpose of this study is to determine the prevalence of human papilloma virus infection and other sexually transmitted infections found in high risk subjects attending our hospital and emphasize on the need of vaccination against HPV in these high risk groups as a part of coordinated strategy to prevent cervical cancer and other HPV related diseases. Biological susceptibility to High risk –HPV acquisition and reduced immune competence for clearance of HR –HPV infection could result from common treatable vaginal infections that disrupt the intricately balanced vaginal ecosystem and its innate protective mechanisms against infection and disease[8]. Hence the identification of concomitant sexually transmitted infections is also done as a part of this study.

AIMS AND OBJECTIVES

The aim of this study is to

1. Determine the prevalence of human papilloma virus infection found in high risk subjects like Female sex workers, Men having sex with men practicing commercial sex, Promiscuous men, Promiscuous women and transgenders practicing commercial sex attending STD clinic in our hospital.

2. To identify concomitant sexually transmitted infections in the study group.

3. Emphasize on the need of vaccination against HPV in these high risk groups as a part of coordinated strategy to prevent cervical cancer and other HPV related diseases.

REVIEW OF LITERATURE

I. GENERAL PROPERTIES OF HUMAN PAPILLOMA VIRUS [9]

The Papilloma viruses are a group of non-enveloped, epitheliotrophic DNA viruses that cause benign lesions of the skin and mucous membrane (wart and condylomas). Papilloma viruses have also been implicated in the development of malignancies of epithelial origin like cancer of the uterine cervix and tumours of the urogenital tract.

The Papillomaviridae family is very large and is currently divided into 16 genera of which five infect humans (Alpha – Beta- Gamma- Mupa, and Nupa papilloma virus). The Papilloma viruses are 55 nm in diameter and contain a larger genome (8 kbp).[10]

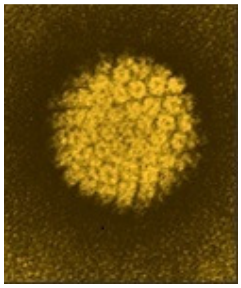
The HPV of greatest medical importance are members of the alpha genus. Beta and gamma species can be commensals as they are frequently isolated from normal skin. The papilloma virus of delta genus are the bovine and ungulate types.

II. VIRION STRUCTURE [9]

Papilloma viruses are small, non enveloped Icasohedral DNA viruses. They replicate in the nucleus of squamous epithelial cells. The papilloma virus

particles have a sedimentation coefficient of 300. The virion particles consist of a single molecule of double stranded circular DNA approx. 8000 base pairs (bp) in size contained within a spherical protein coat or capsid composed of 72 capsomeres. The DNA makes up approximately 12% of the virion by weight. The virus particles have a density in cesium chloride of 1.34 g/ml. The PV capsid consists of two structural proteins. The major capsid protein (L1) has a molecular weight of approximately 55 kd which is approximately 80% of the total viral protein. A minor protein (L2) has a molecular size of approximately 70 Kd. Both the proteins are virally encoded. Virus like particles can be produced from different PVs by expressing L1 alone or the combination of L1 and L2 using mammalian or non mammalian expression systems.

ELECTRON MICROGRAPH IMAGE OF HPV



III. REPLICATION [9,10]

Papilloma virus is highly species specific and highly tropic for squamous epithelial cells of the skin and mucous membranes. The basal cell is the only cell in the squamous epithelium undergoing cell division. The virus must infect the basal cells to establish a persistent lesion. Viral nuclei acid can

be found in basal stem cells but late gene expression (capsid proteins) is restricted to upper most layer of differentiated Keratinocytes. Stages in the viral replicative cycle are dependent on specific factors in sequential differentiated states of epithelial cells. There is a close link of the papilloma virus life cycle with the differentiation of the squamous epithelium. Late gene expression, synthesis of capsid proteins, vegetative viral DNA synthesis and assembly of virions occur only in terminally differentiating squamous epithelial cells.

HPV Genome [11]

The Genomic organization of all HPV types is similar. Three regions can be identified.

1. A small non-coding region of 400 – 1000 base pairs that plays a regulatory role in DNA transcription and replication.
2. An early region (ER) consisting of several genes (E1-E7) coding regulatory proteins in DNA transcription, replication and cell transformations.
3. A Late Region (LR) that codes viral structural proteins L1 and L2.

E1 is a helicase which binds and interacts with host DNA, E2 is involved in recruitment of E1 to the key target region & also has a role in repressing transcription.

E1 –Activates telomerase – promotes continued cell proliferation and inhibits apoptosis. E7 – inactivates the tumour suppressor gene pR6 and allows the cells to enter the S phase.

IV. GEOGRAPHIC DISTRIBUTION [12]

Worldwide differences in the prevalence of genital HPV infection exist and are linked to regional variations in cervical cancer rates. HPV 16, the most common type detected in invasive cervical cancer, has been classified into 5 major lineages viz., Asian variants, American Asian variants, European variants, African 1 variants and African 2 variants. Studies also suggest that variants of HPV 16 and HPV18 to persist in a host where race indicates an ancestral geographic distribution that was once shared with that of the variant. Approximately 291 million women are HPV DNA carriers worldwide. 32% of the HPV infected women have high risk HPV types 16 & 18 from the general population. The median prevalence of high risk HPV by region was Africa (8%), America (11.1%), Eastern Mediterranean (13.7%), Europe (11.5%), South East Asia (13.9%), Western Pacific (6.9%). The prevalence of HPV type 16 ranged from 1.1% to 38.9% across all regions[2]. The most common type reported among female sex workers included HPV 16 (38.9%) and HPV 18 (23.1%)[2].

V. EPIDEMIOLOGY

The incidence and prevalence of clinical HPV genital infection is steadily increasing since the mid 1960's. Current evidence suggests that over

50% of sexually active adults (15- 25 years) have been infected with one or more HPV types. In the United States, the prevalence among men and women between 15 and 49 years of age with genital warts could be 1.4 million and with subclinical infection is 19 million. In Britain and Ireland every 80,000 new cases of anogenital warts are reported every year. The prevalence of anogenital warts in India is reported to be 5.1% to 25.2% of STD patients. HIV infection may increase the incidence and prevalence of genital warts. The incidence of warts is reported between 5% and 27% in HIV Infected individuals. In a prospective study of 912 HIV-1 infected patients, 21% had common / plantars warts and 19% had condyloma acuminata.

Anogenital wart is the most common manifestation of sexually transmissible HPV infection, and prevalence peaks during the second and third decades of life. Prevalence rates of genital warts is between 0.6% and 13% depending on the population studied. Twenty year trends in the incidence and prevalence of diagnosed genital warts in Canada showed an incidence of 149.9/10,000 in men and 170.8/100,000 in women in 1992 and appears to be increasing. Genital warts represent the tip of the iceberg of HPV infection. The spectrum of subclinical infection has several distinct entities namely aceto white epithelium, macroscopically normal epithelium but cytological or histological evidence of HPV infection such as Koilocytosis and macro and microscopically normal epithelium in which HPV has been detected by means like insitu hybridization or DNA amplification by PCR.

The infectivity of patients with warts is well documented. Upto 65% of their sexual partners will develop warts in six months. Genital HPV is less common in circumcised men and cervical cancer risk is less among female sex partners of circumcised men.

Around 70% of genital HPV infections clear within 1 year and 90% in two years. HPV 16 takes longer to be cleared by the immune system than other types having a medium duration of 8 months while it is 3 – 4 months for HPV 6, 11 and 18.

VI. TRANSMISSION OF HPV[14]

Genital HPV infections spread primarily through sexual contact. The infectivity of HPV among sexual partners is around 60%. During sexual intercourse, microabrasions occur in male and female genitalia and anus in Homosexuals. These microabrasions permit the transfer of HPV virions from the epithelial cells of the infected partner to the basal layer of recipient. It is still not proven if moisture and abrasions of the epithelial surface enhance HPV transmission. Transfer by fomites is not known to be responsible in acquiring genital HPV. Digital transmission is known and perinatal transmission has been observed in infants born to women having genital warts during pregnancy. These infants may develop laryngeal papillomas and congenital condylomas. This type of transmission is rare.

VII. PATHOGENESIS [10]

Spread of viral infections occur by close contact. Viral particles are released from the surface of papillomatous lesions and microlesions allow infection of proliferating basal layer cells at other sites or within different hosts. HPV types target epithelial cells. Their replication is dependent on the presence of differentiating squamous epithelium. Viral DNA alone can be detected in the lower layer of the epithelium while capsid (structural) proteins are found in superficial differentiated cell layers. The infectious virus from the epithelial cells enters through the microabrasions caused by trauma of the sexual act and reaches the basal layer of the recipient. In the absence of transformation, HPV follows the normal cycle of reproduction in the basal layer. Clinical and histopathological evidence of HPV infection develops 1-8 months after initial exposure. These lesions regress spontaneously or persist as benign lesions.

VIII. CLINICAL FEATURES[13]

Infection begins with viral entry and follows one of the three paths. Latent infection where the virus remains dormant without producing any or microscopic evidence of disease. Subclinical infection and clinical disease which is both asymptomatic and symptomatic. The lesions appear after an incubation period of 1-8 months with an average of 3 months.

Infections of the genital tract – Genital warts.

In uncircumcised men the preputial cavity (frenulum, glans penis, coronal sulcus, inner aspect of the foreskin) is commonly affected. In circumcised men, the shaft of penis is often involved. Warts also occur on the scrotum, groin, perineum and anal area.

In females, the common sites are the posterior introitus, labia, perineum, and perianal area. Lesions also can be seen intravaginally or in the cervix but these areas are involved in subclinical infection. The urethral meatus is affected in 20-25% males and 4-8% of females. Anal warts are rarely found proximal to the dentate line. Intraanal warts are present when receptive anal intercourse is practiced. The colour of these warts can be pinkish raspberry to salmon red (non –keratinized warts), grayish white (heavily keratinised lesions) and ashen gray to brownish black (Pigmented lesions). Condylomata-tend to be nonpigmented and pigmented lesions are mostly seen on pigmented skin of labia majora, penile shaft, pubis, groin, perineum and anal areas.

IX. CLINICAL TYPES OF GENITAL WARTS[13]

1. Condyloma Acuminata :

The lesions are soft, pink, pedunculated, papilliferous, cauliflower like masses with finger like peduncles and irregular surface. They are mostly seen on moist partially keratinized epithelium such as the preputial cavity, urinary meatus, labia minora, introitus, vagina, cervix, anus and anal canal. They may also affect intertriginous areas as well (groin, perineum and anal area).

Giant condyloma (Buschke – Lownenstein tumour)

This is a very rare variant of HPV 6 and 11 clinical manifestation characterized by aggressive downgrowth into the underlying dermal structures. The other types include Papular warts, verruca vulgaris type or keratotic warts, sessile warts and flat warts.

2. Intra epithelial neoplasia.

Bowenoid papulosis (BP) and Bowens disease (BD) are visible lesion associated with oncogenic HPV types commonly HPV 16, that exhibits full thickness intraepithelial neoplasia (IN–III). BP appears at 25-35 years and BD at 40-50 years or older. BP presents as multiple, small, verrucous or velvetty often pigmented papules involving the anogenital region.

X. SUB CLINICAL INFECTION

Subclinical HPV infections and latent infection are the most likely outcome after exposure to HPV. They are associated with symptoms such as itching, burning, fissuring and dyspareunia. Mucosal surface looks normal until acetic acid is applied, after which well demarcated, round white lesions may appear. In men, an entity called papilloma virus associated balanoposthitis is proposed. The clinical features include recurrent, painful fissuring of the frenulum, the coronal sulcus or the prepuce causing dyspareunia. In women, lesions are often found on the vulva, perineal and perianal area but seldom

around the urethral meatus. Treatment is offered to symptomatic patients. However, subclinical infections are largely asymptomatic.

Atypical lesions

They are brownish red or hyperkeratotic macules clinically identifiable by the naked eye without acetic acid application. Atypical lesions are found near clinically apparent lesions.

Physical and psychosexual implications

Symptoms include inflammation, fissuring itching, bleeding or dyspareunia. Clinical lesions are disfiguring and can affect sexual life style. They cause feelings of guilt, anger, anxiety and loss of self esteem, and create concerns about future fertility and of cancer risk.

Anogenital warts and pregnancy

Warts flourish in pregnancy and there is an increase in size and number of the lesions. This may be due to the increased hormone levels, vascularity and immune deficiency which are seen in pregnancy. Large warts may cause dystocia. Elective LSCS is preferred to prevent transmission of infection to the neonate. Even without treatment warts may resolve after delivery.

Anogential warts in children

Genital warts in children may result from several modes of transmission such as acquisition at birth by HPV transmission from the maternal genital tract, autoinoculation from finger warts, and non sexual transmission from family. Genital warts present at delivery are associated with a risk of 1 in 400 of developing juvenile laryngeal papillomatosis.

Anogenital warts and Immunosuppression

Immunosuppression as a consequence of HIV infection and iatrogenically as a result of transplant grafting is linked to a significant increase in multicentric and refractory condylomata and in intraepithelial neoplasia. Hence annual cytological screening of HIV positive and allografted women is advised.

Genital HPV infection and cancer

Cervical cancer is the second most common cause of death from cancer, after breast cancer among women worldwide. The association between HPV infection and cervical cancer was first suggested in the 1970's. Several epidemiological and molecular studies provided extremely strong evidence for the role of HPV as an etiological agent in cervical cancer and other anal and genital tract malignancies.

Over 99% of cervical cancer and over 80% of anal cancer cases are linked to genital infections with HPV. Anal cancer is associated with high risk

HPV infection. Immunocompromised patients, Men who have sex with men, and HIV infected men are especially at risk.

Anal HPV infection is sexually transmitted in most instances and a history of receptive anal intercourse in women and of homosexual activity in men is associated with an increased risk of this cancer. The risk of anal cancer is much lower in the general population. Most anal cancers arise in the transition zone between columnar & squamous epithelium.

Head and neck cancers

HPV associated cancers are located in the oropharynx including tonsils, tonsillar fossae, base of the tongue and soft palate. HPV positive tumours have a characteristic basaloid morphology and genital-oral sex may be a risk factor for these tumors.

Penile Intraepithelial neoplasia, Vulval intraepithelial neoplasia and Anal intra epithelial neoplasia (AIN).[15]

These squamous intraepithelial lesions (SIL) are divided into three basic types.

(1) Subclinical, appearing only following the application of acetic acid
(2) Resembling flat warts
(3) Thirdly and most distinctive red velvetty resions which have an eroded appearance because there is no normal epithelium.

SIL are classed as low grade or grades (1) and high or grade (2) or (3).

Majority of low grade lesions being associated with non oncogenic HPV type 6 and 11 and majority of grade 3 lesions being caused by oncogenic HPV types, predominantly 16 and 18. Low grade lesions rarely show malignant potential whereas high grade lesions undergo malignant change.

Recurrent largyngeal papillomatosis [9]

Laryngeal papillomatosis is rare. The papillomas may severely compromise, the airway particularly in young children. The vocal cords of the larynx is the most common site affected but papillomas can arise at other sites like trachea, lungs, nose and oral cavity. Most lesions are caused by genital HPV types causing external genital warts especially types 6 and 11. RRP(Recurrent Respiratory Papillomatosis) occur both in children and adults. Most cases of RRP that arise in early childhood are attributable to intrapartum transmission of HPV. A history of oro genital sex is a risk factor in adult RRP. RRP is associated with a low risk of spread to the bronchi, and lungs, progression to severe dysplasia and even to cancer.

Oncogenic types [13]

HPV associated with anogenital infections are classified based on their oncogenic risk into the following:

Low or no oncogenic risk – HPV types 6, 11, 42, 43 and 44.

Intermediate risk- HPV types 31, 33, 35, 51, and 52 and

High risk- HPV types 16, 18, 45, 46.

More than 20 types of HPV are associated with cervical cancer. HPV 16 is most commonly linked with cancer since it is present in 50% of cervical carcinomas. HPV type 18 is a very high risk type and its association with cervical lesions has a poor prognosis. Apart from cervical carcinoma, oncogenic HPV have been detected in other anogenital cancers. Bowenoid papulosis (HPV types 16, 34, 37, 42) penile carcimoma (HPV types 16 and 18) and anal carcinoma (HPV 16, 18 and 33).

MECHANISM OF ONCOGENESIS[13]

Infection with HPV is one step in the multifactorial causation of malignancy. DNA from HPV types that are commonly found in anogenital cancer alters the growth of cells they infect, in a way that converts them to immortalized cell lines capable of continued replication. In benign HPV associated lesions, the viral genome exists separate from the host cell DNA replicating as an extrachromosomal plasmid. In malignant lesions associated with HPV16 and 18, the viral DNA is integrated into the host genome. Integration in to host DNA involves breakup of the viral genome. This results in the loss of function of the early E1/E2 regulatory genes associated with replication and transcription of the virus and a deregulation of the E6/E7 genes associated with cellular transformation. E6/E7 genes of the virus possibly cause

transformation of HPV infected cells by interfering with the function of the P53 protein (E6) and the Rb retinoblastoma gene product (E7). Both these proteins are termed as `anti-oncogenes' as they play a role in preventing cellular transformation by suppressing cellular division and proliferation.

Genital and related lesion associated with HPV[15]

Lesions	-	Predominant HPV types
Condyloma acuminata	-	6, 11
Buschke – Lowenstein tumour	-	6, 11
Recurrent respiratory papillamatosis	-	6, 11
Carcinoma of head, neck and lung	-	16, 18, 30
Oral papilloma	-	6, 7, 11, 16, 13 (72, 73 also in HIV patients)
Cacinoma of cervix	-	16 (55%) 18 (16%) global averages (WHO Data) Predominant minor types : 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66
CIN 1	-	Compared with cervical cancer prevalence of 16/18 is halved and prevalence of minor types is higher (WHO data)

CIN 2/3	-	16 (42%) 18(7%) 31 (7%) 58 (8%) WHO data
Carcinoma of penis	-	16 (59%) Predominant minor types 18, 35, 45
Carcinoma of anus	-	16 (87%), 18 (7%), 33 (6%), 31(1%)
Other types occasionally associated with malignancy	-	40, 42-44, 53, 54, 59, 61, 68, 70, 72, 73, 81, 82

IMMUNOLOGY OF WARTS

Inability to propagate HPV in the laboratory is a roadblock on the investigation on the immunology of warts. Both cell mediated and humoral immunity have been documented in patient with genital warts but CMI appears to be important. Increase in the CMI has been shown to be effective in regression, controlling reactivation, prevention of recurrence and elimination of warts. Patients with deficient CMI (HPV patients, renal transplant patients receiving immunosuppressive drugs) have increased incidence of warts.

Various studies show that human sera have antibodies that reacts to HPV protein in patients with regressinig warts. IgM (100%) IgG (97%) and IgA (80%) classes of antibodies to HPV antigens have been detected. In 83% of these patients IgM class of antibodies to virus infected cells are also found. Patients with complement fixing IgG antibodies had a higher cure rate and a shorter duration of warts (less than a year). Though circulating antibodies are

detected in patients with warts, they do not help in clearance of the lesion or in the prevention of recurrences.

CLINICAL DIAGNOSIS / INVESTIGATIONS [13]

The diagnosis of anogenital warts is based on the history of exposure, clinical appearance and epidemiological proof of the warts in the sexual contact. In both sexes careful examination of the external genitalia is performed with a clear and powerful light. All women with anogenital warts should have a speculum examination. It is essential to look for concurrent STDs and tests for them should be offered.

THE ACETIC ACID TEST

Following the application of 3%-5% acetic acid, the HPV lesions turn grayish white. The whitish appearance is attributed to an over expression of cytokeratin 10 in the HPV infected suprabasal cells. The test has low specificity and sensitivity and not recommended for screening purposes. It can be used for visualizing subclinical genital HPV associated lesions.

COLPOSCOPY

Higher sensitivity for low grade CIN

MEATOSCOPY

Inspection of fossa navicularis in men is done by using a small speculum.

PROCTOSCOPY

Concurrent perineal and perianal warts exist in 1/3rd of patients. Hence proctoscopy is indicated.

SOUTHERN BLOT HYBRIDIZATION

This technique is the Gold Standard among HPV DNA detection methods with a sensitivity range between 0.1 and 0.01 viral copies per cell but it is too time consuming and labour intensive for routine diagnosis.

IN SITU HYBRIDIZATION

It helps in determining the localization of HPV DNA, within the specimen. Biotinylated probes are more stable and do not expose the staff to radioactivity.

POLYMERASE CHAIN REACTION

This is the most sensitive method for detection of HPV DNA, being able to detect one viral genome in 1,00,000 cells. It is able to detect latent infection but has little benefit in routine diagnosis and management and is primarily a research tool.

HISTOPATHOLOGICAL EXAMINATION

Parakeratotichyperkeratosis, moderate granulomatosis, pronounced acanthosis, papillomatosis & presence of mitotic figures. The characteristic features important for diagnosis are the presence of koilocytes which are mature squamous cells with a large, clear perinuclear zone and smudgy nuclei scattered throughout the outer cell layers.

TREATMENT

The three main therapeutic goals are

1. Induction of wart free periods to allieviate anxiety and to reduce the risk of transmission of HPV.
2. Therapy that is no worse than the disease.
3. Reduce morbidity and mortality for cervical cancer.

Treatment options may be categorized as

Cyto destructive methods

Surgical excision

Cryotherapy

Laser therapy

Bichloracetic or trichloracetic acid

Podophyllin

Podophyllotoxin

Anti metabolite therapy (5- FU)

Antiviral therapy (Cidofivir and interferons) (IFN's)

Immunomodulation (imiquimod)

They have also been classified as

Home therapy: podophyllotoxin (0.5 solution or 0.15% cream) and imiquimod

Hospital therapy: Electrosurgery, laser ablation curettage or scissors excision, cryotherapy, TCA or carbolic acid and Podophyllin.

Therapies generally not recommended IFNs and 5-FU.

PODOPHYLLIN

It is used as 10% to 25% solution dissolved in tincture of benzoin. It is a first line therapy and cheap with common clinical use due to spectacular initial effects. Ethanol extract is prepared from the dried rhizome and roots of an American plant podophyllum peltatum and P.emodi, an Indian Plant which is a complex resinous material containing podophyllotoxin, alpha peltatum and betapeltatum.

Podophyllin inhibits mitosis at metaphase and causes swelling & necrosis of cells..

Applied to the warts by a physician using a cotton tipped swab once or twice a week for up to six weeks. The surrounding skin is protected with Vaseline, zinc cream or both. 4 hrs after application it is completely washed off. After six sittings, if the warts persist other treatment modalities need to be considered.

The wart clearance rate at the end of treatment is 32%-79%.Podophyllin is contraindicated in pregnancy as it can lead to fetal death and abortions. Podophyllin – prolonged use is not advocated for the fear of its oncogenic potential due to its two chemical mutagens (quercetin and kaempherol).

COURSE AND PROGNOSIS

The natural course of genital warts spans from limited easily treatable disease to extensive progressive disease resulting in intraepithelial neoplasia or invasive squamous cell carcinoma.

PREVENTION AND CONTROL[10,14,15]

Vaccines against HPV are expected to be a cost effective way to reduce anogenital HPV infections, the incidence of cervical cancer and the HPV associated health care burden. A quadrivalent HPV vaccine was approved in the United States in 2006 and a bivalent vaccine in 2007. Both are noninfectious recombinant vaccines containing virus like particles composed of HPVL1 proteins. The Quadrivalent vaccine contains particles derived from

HPV types 6, 11, 16, 18 (Gardasil) and bivalent vaccine contains VLP's derived from HPV types 16 and 18 (cervarix - GSK).

The mode of action of these vaccines is thought to be the induction and production of anti-L1 antibodies. The VLP's are potent immunogens and given intramuscularly which ensures rapid dissemination of the antigens to the lymph nodes. This results in high levels of neutralizing antibodies, which are able to bind to virus and prevent entry and infection of cells. Both vaccines are effective at preventing persistent infections by the targeted HPV types and the development of HPV related genital precancerous lesions. The striking features of these 2 vacccines has been the near 100% protection from clinical diseases such as high grade CIN2/3 which are accepted as the precursor lesions of malignant transformation. Both vaccines will protect against approximately 70% of cervical cancer cases. The vaccines have provided a degree of cross protection against subtypes not included in the vaccine such as type 45 and 31. The current vaccines are expected to offer a degree of protection against all HPV related cancers such as other anogenital cancers (ie anal, vulval, vaginal, penile etc.) and subsets of head and neck cancers in which the dominant sub types are HPV 16 and 18. The quadrivalent vaccine will additionally protect against the development of genital warts and recurrent respiratory papillomatosis.

The HPV vaccine is recommended for 11–12 year old girls (range 9–26 years). Ideally the vaccine is administered before the onset of sexual

activity.Young men, especially men who have sex with men (MSM) who carry a significant risk of infection should also be vaccinated. Men could be protected against both warts and HPV related cancers and their vaccination would also benefit herd immunity.

The HPV vaccine has been tested in over 11,000 females (9-26 years of age) in many countries around the world. These studies found that HPV vaccines were safe and caused no serious side effects.

The vaccine is not effective against established disease and are not recommended for pregnant females. The vaccine induced immunity lasts for 5 years.

The vaccine should be delivered through a series of three intramuscular injections over a six month period. The second and third doses should be given 2 and 6 months after the first dose.

HPV VACCINE EFFICACY

The clinical trials have demonstrated 100% efficacy in preventing cervical precancers caused by the targeted HPV types and nearly 100% efficacy in preventing Vulvar and vaginal precancers and genital warts caused by the targeted HPV types.

HPV AND HIV INTERACTION [9]

The risk of genital HPV infection may be high for HIV infected individuals because infection by both viruses is often through sexual transmission and it is well known that exposure to one sexually transmitted agent puts an individual at higher risk for exposure to other sexually transmitted agents. Cervical HPV infection is detected more frequently in HIV positive women than in those who are HIV negative. The infection is likely to persist, result in cytologic abnormalities and a much greater risk is seen for high grade dysplasia. In a prospective study of women at high risk for HPV infection in New York, cervical swabs taken at the initial examination detected HPV DNA approximately twice as frequently in HIV positive women compared with HIV negative women (56% versus 31%) persistent infection was 6 times as frequent in the HIV positive women (24% versus 4%), Low grade dysplasia (C1N1) was about three times as frequent in HIV positive women (13% versus 4%) whereas high grade dysplasia was seven times more frequent (7% vs 1%). Low CD4 counts in HIV positive women represent an independent risk factor.

HIV infection represents a risk factor for other malignancies associated with HPV infection including cancers of the vulva, penis and anus. The relative risk for anal cancer is especially high for HIV infected male homosexuals.

Studies have reported that 26% to 60% of HIV seropositive men and 15% to 29% of seronegative men have anal HPV DNA.

MECHANISMS OF INTERACTIONS BETWEEN HIV AND HPV[14]

Alterations in the natural history of HPV infection and of HPV related neoplasia among HIV seropositive individuals are the result of general or local HIV induced immunesystem dysfunction. Control of HPV is improved when large number of lymphocytes or Langerhans cells in the area are infected with HIV. It is also possible that HIV act directly on HPV. In vitro studies have shown that intracellular HIV 1 tat m RNA can transactivate HPV type 16 E6 & E7 a step that is important in development of squamous cell neoplasia. In vitro studies have also shown that extracellular HIV-1 tat protein can enter HPV infected cells and upregulate HPV type 16 E6 and E7. The HIV-1 tat protein enhances E2 dependent HPV type 16 transcription. Extracellular tat migrates from Langerhans cells or other HIV infected mononuclear cells that abut HPV infected epithelial cells and upregulate HPV.

TYPES OF COMMON SEXUALLY TRANSMITTED INFECTIONS[16,17]

BACTERIAL	**PARASITIC**
Gonorrhoea	Trichomoniasis
Bacterial vaginosis	
Syphilis	

VIRAL	**FUNGAL**
Human Papilloma Virus	Candidiasis
Human Immuno deficiency virus	

Herpes Genitalis

Hepatitis B and C virus

GONORRHOEA

Niesseria Gonorrhoeae is a gram negative intracellular diplococci which affects the mucous membrane of the genito urinary tract, eye, rectum and throat causing acute suppuration, tissue invasion followed by chronic inflammation and fibrosis. In males it causes urethritis and in females it affects the endocervix producing mucopurulent discharge. Gram stained smears of the endocervix or the urethral exudates shows many intracellular gram negative diplococci within pus cells. Stained smears from urethral exudates in men have a sensitivity of about 90% and a specificity of about 99%. In women, stained smears from endocervical exudates have a sensitivity of about 50 % and a specificity of about 95%. Cultures of urethral exudates from men are not necessary when the gram stain is positive but cultures are done for women.

BACTERIAL VAGINOSIS

It is a clinical syndrome with the symptoms of increased vaginal discharge with foul smelling fishy odour which is more offensive after sexual intercourse. Gardnerella Vaginalis, Prevotella spp, Peptostreptococci, Mobiluncus spp, Mycoplasma hominis are closely associated wth bacterial vaginosis. The accepted standard for the diagnosis of bacterial vaginosis has been the Amsel's criteria(thin homogenous vaginal discharge, increased vaginal pH, presence of amines detected by fishy odour after addition of KOH

to the discharge and the presence of clue cells). Alternative to this method is the Nugent's scoring in which the grading or scoring of the gram stained vaginal smear to detect the changes in vaginal ecology.

SYPHILIS

Treponema Pallidum, the causative agent of syphilis is actively motile slender spirochaete which can be seen only under dark ground illumination. Natural infection with T.Pallidum is restricted to human host and transmitted by sexual contact. Both the primary lesion(hard chancre) and the secondary lesion(rash and condyloma) are highly infectious and rich in spirochaetes. Laboratory diagnosis is by examination of tissue fluid for motile spirochaetes by dark field microscope and serological tests which are non specific (RPR) and specific (TPHA, FTABS, TPI tests).

TRICHOMONIASIS

Trichomonas Vaginalis is a pear shaped parasite affecting both man and women, transmitted commonly by sexual intercourse. In females the infection is limited to vulva, vagina or cervix. In males urethra, prostrate and seminal vesicles may be infected. Females tend to have a profuse frothy yellow discharge while males have a thin urethral discharge which shows characteristic motile trichomanads in wobbling or rotating motion.

CANDIDIASIS

Candidial vulvovaginitis is the second most frequent cause of vaginal infection more common in immunocompromised and diabetic patients. Patient presents with thick white vaginal discharge. Microscopic examination of KOH mount and Gram stain from the discharge shows budding yeast cells and pseudohyphae .

HIV

Human immunodeficiency virus is an enveloped RNA virus belonging to the lentivirus subgroup of retroviruses. HIV is present in semen, vaginal and cervical secretions and blood, the main vehicle by which the virus is transmitted. HIV is mainly transmitted by heterosexual intercourse(85 to 95%) transmission, anal intercourse, vertical transmission, transfusion of unscreened blood, use of contaminated syringes and needles and by intravenous drug users. HIV screening is done by rapid HIV antibody tests which are highly sensitive and specific. ELISA (Enzyme Linked ImmunoSorbent Assay) for HIV antibody screening is done in blood transfusion centres and for surveillance testing.

HERPES GENITALIS

Herpes simplex virus is an alpha herpes virus among the herpes virus family. It is fast growing, cytolytic and have latent infection in neurons of the host. Genital herpes is predominantly caused by human herpes virus 2 and also

by human herpes virus1. It affects the mucosal surfaces and damaged cutaneous sites. The lesions are Vesiculo ulcerative in the penis and vesiculo ulcerative lesions of the cervix, vagina, vulva and perineum in females and are painful associated with fever, malaise, dysuria and inguinal lymphadenopathy. Laboratory diagnosis include skin scrapings obtained from the base of the vesicle stained with Giemsa stain will show the multinucleate giant cells (TZANCK SMEAR) which is indicative of herpes virus. The virus can be isolated from CSF, throat, urine, stool, nasopharynx and conjunctiva by PCR and serological assay can be done to detect antibodies.

MATERIALS AND METHODS

PLACE OF STUDY

(1) Department of Microbiology, Stanley medical college, Chennai

(2) Department of Sexually Transmitted Diseases' Stanley medical college and Hospital, Chennai.

STUDY DESIGN

Prospective study

SAMPLE SIZE

200

STUDY PERIOD

September 2014 to September 2015

ETHICAL CONSIDERATION

Ethical and research clearance was obtained from the Ethical committee, Stanley medical college. Permission to conduct the study was sought from the respective hospital department authorities. Personal interview was conducted by the author with every patient. A standard proforma was filled up at the time of interview and Informed consent was obtained from the patients before enrollment into the study.

STATISTICAL ANALYSIS

The collected data was analysed with SPSS 16.0 version. To describe about the data descriptive statistics frequency analysis, percentage analysis were used for categorical variables and the mean & S.D were used for continuous variables. To find the significance in categorical data Chi-Square test was used. In the above statistical tool the probability value .05 is considered as significant level.

INCLUSION CRITERIA

1. Female sex workers [FSW]
2. Male having sex with male [MSM] practicing commercial sex
3. Transgenders [TG] practicing commercial sex
4. Men above 18 years attending STD clinic with history of PROMISCUOUS sexual activity- Having multiple sex partners in one year duration.
5. Women above 18 years attending STD clinic with history of PROMISCUOUS sexual activity- Having multiple sex partners in one year duration.
6. FSW, MSM, TGs, Promiscuous men and women with other symptoms of sexually transmitted diseases.

EXCLUSION CRITERIA

1. Men above 18 years attending STD clinic without history of promiscuous sexual activity.
2. Women above 18 years attending STD clinic without history of promiscuous sexual activity.
3. Pregnant women.

SPECIMEN COLLECTION AND LABORATORY TESTING

SPECIMEN COLLECTION

URETHRAL SWAB[15]

The specimen was collected after wearing sterile gloves one hour after patient has urinated. After retracting the prepuce, the tip of the meatus was cleaned with normal saline and if purulent discharge was seen it was collected using a sterile swab. If no discharge was seen, milking of the urethra was done towards the orifice to obtain the discharge. Three swabs were taken one for gram's stain one for wet mount and another was placed in 70% ethanol in a test tube for viral isolation.

THROAT SWAB[18]

It was made sure that the patient was not treated with antibiotics or antiseptic mouth washes 8 hours before swabbing.

In a good light and using the handle of a tongue depressor the tongue was depressed and inside of the mouth was examined. Swabbing was done in

the posterior pharyngeal wall and peritonsillar area using a sterile cotton wool swab. Two swabs were taken one for gram's stain and another placed in 70% ethanol for viral isolation.

CERVICAL SWAB/HIGH VAGINAL SWAB

Sterile vaginal speculum was used to examine the cervix and the specimen was collected. Speculum was moistened with sterile warm water and inserted in to the vagina. Cervix was cleaned using a swab moistened with sterile physiological saline. A sterile Dacron swab is passed 20mm-30mm in to the endocervical canal and gently rotated against the endocervical wall to obtain a specimen. Three swabs were taken one for gram's stain one for wet mount and one was placed in 70% ethanol in a test tube for viral isolation

COLLECTION OF VAGINAL DISCHARGE[17]

Wet mount to detect motile T. Vaginalis- Using a sterile Dacron swab specimen is collected from the vagina and the exudate is transferred to a microscope slide to which a drop of normal saline is added and mixed and then covered with a cover glass.

Direct smear for Gram staining to detect Candida and examine for clue *Cells*-

Using a sterile Dacron swab specimen was collected from the vagina. Sample of the exudate is transferred to a microscope slide and then spread to make a thin smear. Smear was air dried and heat fixed for gram staining.

KOH mount to detect candida

Using a sterile Dacron swab specimen was collected from vagina. A drop of 10% KOH is placed over the microscope slide. The sample is transferred to the slide and a cover glass is applied over it.

RECTAL SWABS

Specimen was collected using a sterile cotton wool swab. After the insertion of proctoscope, moist swab was introduced in to the rectum for about 2 to 4cm. Swabs were taken avoiding unnecessary contamination of the specimen with bacteria from the anal skin. Swabs with heavy feacal contamination are discarded. Two rectal swabs were taken one for gram's stain and another placed in 70% ethanol for viral isolation.

BLOOD

5ml of venous blood collected from each patient with strict aseptic precautions and serum was separated by keeping the test tube in a 45* slanting position at 4 to 8*c for 4 to 6hours or by centrifuging at 1500 rpm for 5-10 minutes. Then the serum was tested for HIV as per NACO guidelines[19] and RPR for Syphilis was done. The samples that were found to be reactive with RPR are confirmed by performing TPHA test.

PROCESSING OF THE COLLECTED SPECIMEN

Neisseria gonorrhoea

Microscopy

With one swab, on a clean grease free slide, smear was prepared by rolling the swab. One directional smearing technique minimizes distortion and breakage of Polymorphonuclear leukocytes (PMNL) and thereby preserves the characteristic intracellular appearance of this microorganism. The smear was air dried and methanol fixed. Gram stain was done using saffranin as counterstain and examined with light microscope under oil immersion objective.

Interpretation

The smear was examined for epithelial cells, Polymorphonuclear leucocytes (pus cells), organisms and their location whether extracellular or intracellular. The gonococci are intracellular; bean shaped and are usually arranged in pairs, 0.8μm x 0.6μm in size. They are Gram negative in reaction and are stained pink along with the nuclei and protoplasm of pus cells. The slide was examined for at least 2 minutes before declaring as negative for gonococci.

Trichomonas vaginalis

Microscopy

A drop of discharge or urine sediment was put on a clean grease free microscope slide. One drop of normal saline was mixed with sediment and a

cover slip was placed over it. The slide was observed first under 10 x magnification. Any field which shows the suspected organism is then seen under 40 x magnification of light microscope.

Interpretation

Trichomonads are 15 μm in size, they have a pyriform shape with an anterior tuft of flagella and a lateral undulating membrane, the parasite moves actively, showing jerky motility, Centrifuged samples do not show motility because the flagella are detached during centrifugation.

Stainings

On a clean grease free microscope slide smear was prepared from sediment. Then the smear was allowed to dry in air, fixed with methanol and stained with Giemsa stain. Stained smear was observed under oil immersion.

Candida species

KOH MOUNT (10%) FOR CANDIDA

Place a drop of 10% KOH (Potassium hydroxide) on a microscope slide. Transfer the specimen to the slide and apply a cover glass over it.KOH is a strong alkali and hence it clears the epithelial cells ,and digests the keratin around the fungi so that the fungal elements can be seen clearly.Clearing can be hastened by gently heating the preparation over the flame of a Bunsen burner taking care not to prevent drying or splatter of corrosive KOH solution. Examine the slide under 10x and 40x of the light microscope.

Budding yeast cells and pseudohyphae can be seen.

Gram Staining

A smear was prepared on a clean microscope slide, air dried and heat fixed and stained by Gram's Method and observed under oil immersion.

Interpretation

Gram positive budding yeast cells and pseudohyphae**.**

BACTERIAL VAGINOSIS[16,20]

The collection of material is done using a speculum. Speculum examination is made and the nature of the discharge is evaluated. Specimen from the lateral vaginal wall and posterior fornix is taken with a sterile swab. The classical BV discharge is thin, homogeneous and grey/yellow in color while the absence of the classic discharge does not rule out disturbed vaginal flora.

The diagnostic methods used are either laboratory based or clinical 'bedside' testing. For the purposes of laboratory based testing the swab was rolled across a slide and the material allowed to air dry. In the laboratory, the smear should be heat-fixed and Gram-stained.

In the methodology by Nugent et al, the swab was obtained from the lateral vaginal wall and rolled on a glass slide. The smears were then heat fixed and Gram stained using safranin as the counterstain. The smear was then evaluated for the following morphotypes under oil immersion

(1000xmagnification): large Gram-positive rods (lactobacillus morphotypes), small Gram-variable rods (*G vaginalis*morphotypes), small Gram-negative rods

(*Bacteroides* species morphotypes), curved Gram-variable rods (*Mobiluncus* species morphotypes) and Gram-positive cocci. Although Gram-positive cocci are not part of the scoring system, some laboratories will report them if they are present in significant numbers. Increased numbers of Gram-positive cocci are not part of the pattern of the normal vaginal flora. A score of zero to three is considered to be normal, four to six is considered intermediate and seven to ten is defined as Bacterial Vaginosis. Intermediate vaginal flora is reported to the clinician for management based on the clinical context. Thirty two per cent of patients with an intermediate score will proceed to BV and 30% to normal flora. Even though clinical methodology is useful because it allows for an immediate answer in certain urgent clinical situations, but the Gram stain method appears to be more accurate.

SL No	Organism Morphotypes	Number/ oil immersion field	Score
1	Lactobacillis-like (parallel-sided, gram positive rods)	>30 5-30 1-4 <1 0	0 1 2 3 4
2	Mobiluncus–like (curved, gram negative rods)	>5 1-4 0	2 1 0
3	Gardernerella/bacteriods like(tiny, gram variable coccobacilli and rounded, pleomorphic, gram negative rods with vacuole)	>30 5-30 1-4 <1 0	4 3 2 1 0

0-3 normal
4-6 intermediate, test to be repeated later
7-10 bacterial vaginosis

GENITAL ULCER SYNDROME

The exudates from the base of the ulcer is examined by making a wet mount to detect spirochaetes under dark ground illumination, Tzanck smear (1% aqueous toluidine blue) to look for multinucleated giant cells and gram stain done to detect other organisms.

ISOLATION OF CELLS FROM THE SWAB

The swab placed in 70% ethanol in a test tube is vortex mixed to loosen the cells in the swab and then the swab was discarded. The test tube containing 70% ethanol and the collected cells is centrifuged at 8000rpm for 1minute so that the cells get deposited at the bottom of the tube.The 70% ethanol is discarded leaving the celldeposits in the tube to which 0.5ml of PBS buffer is added. The cell deposit is transferred to a 1.5ml microcentrifuge tube for DNA isolation.

POLYMERASE CHAIN REACTION- ISOLATION OF VIRAL NUCLEIC ACID [21]

PRINCIPLE

Cells are lysed during a short incubation with choatrophic salt,which immediately inactivates allnucleases. Cellular nucleic acids bind selectively to special glass filters prepacked in the purification filter tubes. Bound nucleic

acids are purified in a series of rapid wash and spin steps to remove the contaminating cellular components.A special inhibitor removal buffer has been included which removes inhibitors from the preparation. Finally elution buffer releases nucleic acids from the glass fiber. This simple method eliminates the need for the organic solvent extractions and nucleic acid precipitation allowing for purification of many samples simultaneously.

MATERIALS REQUIRED

1. Micropipettes of variable volume 0.5-10, 10-100,and 100-1000.
2. Sterile pipette tips with aerosol barrier 2-20,10-100 and 100-1000.
3. Disposable powder free gloves.
4. Vortex mixer/ water bath
5. Centrifuge with rotor for 1.5ml reaction tubes.
6. 1.5ml/2ml centrifuge tubes.

PROCEDURE

Before use all the reagents were thawed completely mixed and centrifuged briefly.

1. The following reagents areadded to a nuclease free 1.5 ml centrifuge tube.
    a. 200microlitreof lysis buffer
    b. 5microlitre of carrier RNA
    c. 200microlitre of cell deposit
    d. 20 microlitre of Proteinase K
    e. 5 microlitre of internal template control.

2. Centrifuge tube was mixed immediately by inverting it.

3. The tube is incubated for 15 minutes @56*in a water bath.

4. After taking the tube from the incubator 300µlitres of 100% ethanol was added and mixed by a vortex mixer for 30 seconds.

5. Then the tube was centrifuged for few seconds to bring down the drops to the bottom of the tube.

6. Entire sample was then transferred to a pure fast spin column.

7. The pure fast spin column was centrifuged at 12000rpm for one minute.

8. The flow through was discarded and column was placed back into the same collection tube.

9. To this 500microlitre of 70% ethanol was added to the pure fast spin column.

10. The pure fast spin column was centrifuged at 12000 rpm for 1 minute and the flow through was discarded and column was placed back in to the same collection tube.

11. The empty spin column was attached with the collection tube and centrifuged at 12000 rpm for an additional 2 minutes to avoid residual ethanol and the collection tube was discarded.

12. The pure fast spin column was then transferred to a fresh 1.5ml micro centrifuge tube. To this 50 microlitre of elution buffer was added to the

centre of the pure fast spin column membrane and incubated for 2minutes at room temperature.

13. The pure fast spin column and the centrifuge tube was centrifuged at 12000 rpm for 1minute and the pure fast spin column was then discarded.

14. Now the centrifuge tube containing the eluted nucleic acid was stored at -80*c for later analysis.

PREPARATION OF AGAROSE GEL

2% Agarose gel was prepared by adding two grams of agarose powder to 100ml of electrophoresis buffer, then heated in a microwave oven for 3 minutes and mixed until the agarose was evenly dissolved. After cooling to about 60*c ,5microlitre of ethidium bromide was added to 100ml of the gel to enable visualization of DNA after electrophoresis. Ethidium bromide being carcinogen should be handled with precaution.

A well formed COMB plate was placed across the end of the casting tray which is covered with a cellophane tape in its both ends and the freshly prepared gel was poured into the casting tray which acts as a mold. This was let to solidify at room temperature.

PREPARATION OF ELECTROPHORETIC BUFFER

Electrophoresis [TAE- Tris Acetic Acid-EDTA] buffer was prepared in a 50 X dilution. To prepare a 1000ml of buffer , 980ml of distilled water and 20

ml of buffer is added and poured in to a electrophoretic tank. After the gel has hardened enough, the gel was gently placed on the electrophoresis tank with buffer and was made sure that the gel was completely immersed in the buffer.

MULTIPLEX POLYMERASE CHAIN REACTION FOR HUMAN PAPILLOMA VIRUS 16 AND 18

The kit components include PCR master mix, HPV 16 & 18 primer mix, endogenous primer mix, HPV 16 & 18 positive template and nuclease free water. The kit contains all reagents and enzymes for the specific amplification of E6/ E7 region of the HPV genome. In addition it contains an endogenous control system to identify possible PCR inhibition and to validate DNA purification.

MASTER MIX

Master mix is prepared taking 10µl of master mix and 10µl of HPV 16 and 18 PRIMER MIX for one sample and mixed together. 20µl of this mixture is added to a 0.2 ml PCR tube to which 5µl of sample is added. Endogenous control, positive and negative controls are also added in the similar method.

The primer sequence of HPV 16 and HPV 18 used in this procedure are:

HPV 16

5’-GCAACAGTTA CTGCGACGTGAGGT-3’

5’-CACACAA CGGTTTGTTGTATTGCTGT-3’

HPV 18

5’-TGATCTGTGCACGGAACTGAACAC-3’

5,-TCAACGGTTTCTGGCACCGCAG-3’

THERMOCYCLING

The 0.2ml tubes having the positive and negative controls, endogenous control and the sample are placed in the thermocycler and the programme has been set to run in the following steps

1. Denaturation at 95*c for 30 seconds

2. Annealing at 54*c for 30 seconds

3. Primer extension at 72*c for 30 seconds

4. Again step 1, the cycle is repeated for 35 times.

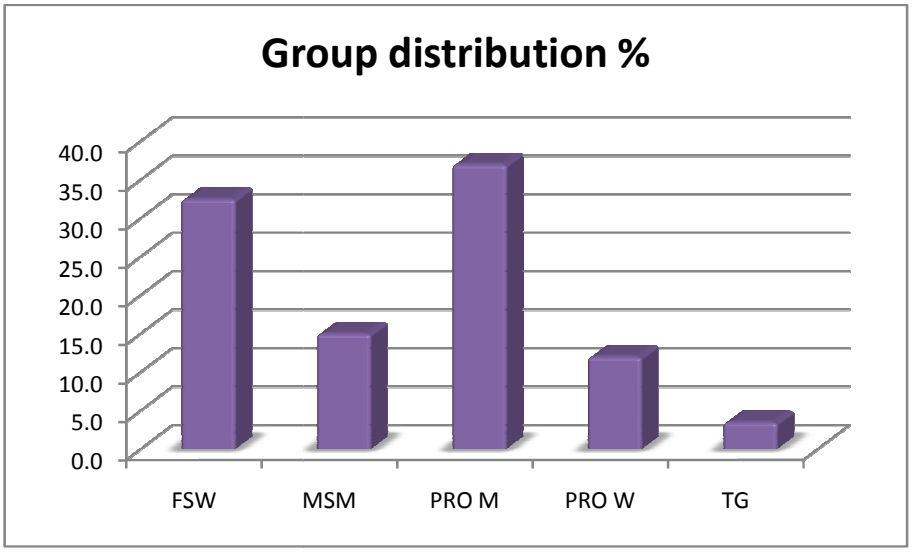
GEL ELECTROPHORESIS

After the amplification cycles [35 cycles] are completed in the thermocycler, the tubes are taken out from the thermocycler and then 5µl of DNA ladder, 20µl of sample and 20µl negative, positive and endogenous controls, are loaded in to gel wells using micropipette. Electrical leads are connected to the electrophoresis tank. Current is supplied with a voltage of 150 v so that negatively charged DNA migrate from cathode to anode. Gel running time is approximately the time taken by the gel loading dye to cover ¾ ths of distance in the gel.

After the gel electrophoresis, PCR products are observed using UV transilluminator. The DNA ladder is a mixture of fragments with known size to compare with the PCR fragments. Amplicons of size 200bp and 300bp are consistent with HPV 16 E7 and HPV 18 E7 respectively and are taken as positive.

OBSERVATION AND RESULTS

Two hundred high risk subjects [female sex workers FSW-65, Male having sex with male MSM-30, Promiscuous men PRO M-74, Promiscuous women PRO W-24, and Transgenders TG-7] who attended STD clinic were recruited and included in the study.



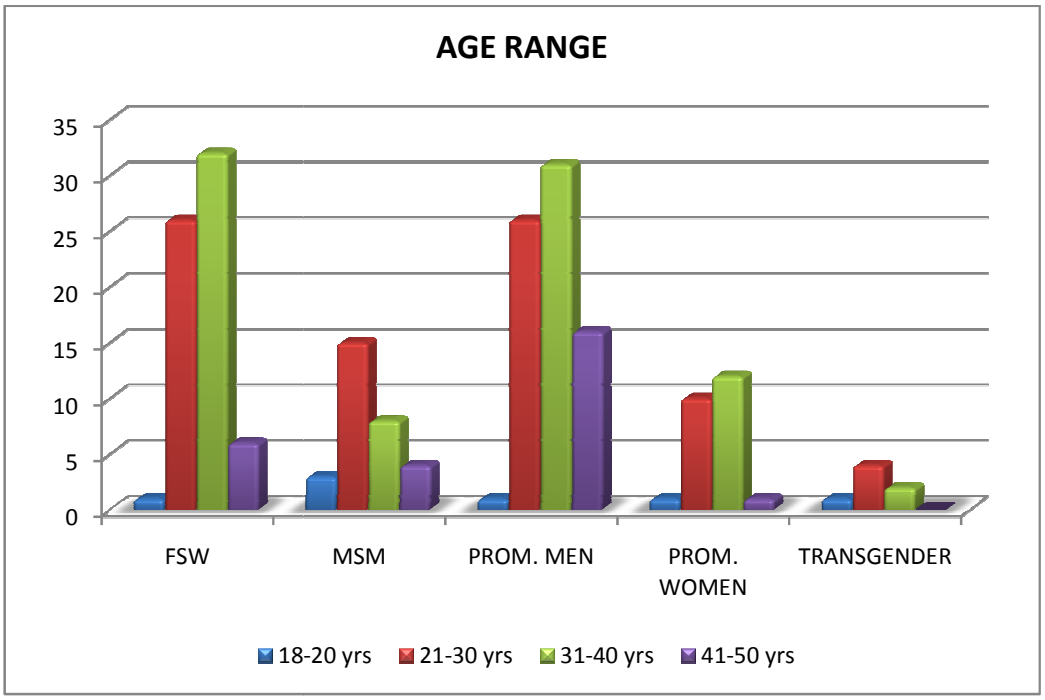
Samples for HPV 16 & 18 viral isolation were taken according to the sexual habits of the patients and blood for serological testing for syphilis and HIV was collected from all the patients after obtaining their consent for the same.

Specimens taken included pharyngeal, rectal, high vaginal, endocervical and urethral swabs. All the specimens were processed in the Department of Microbiology, at our institute. The results obtained were analysed.

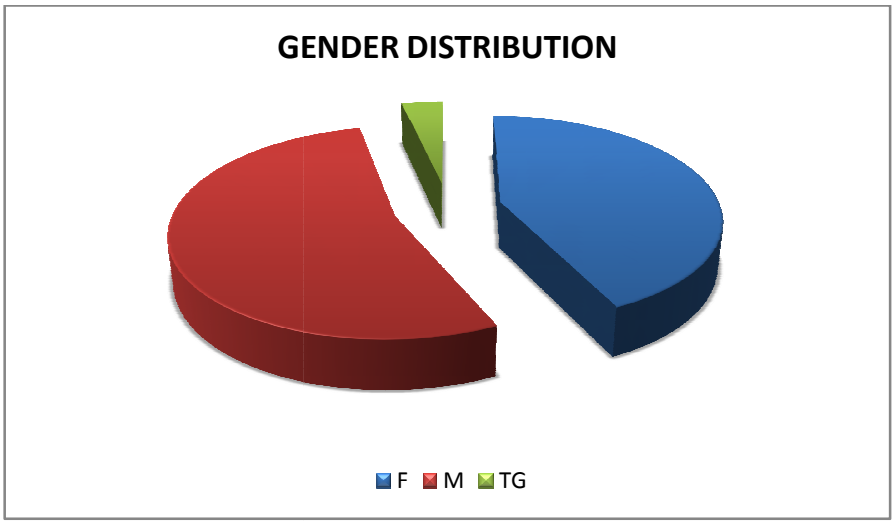
AGE OF STUDY POPULATION- TABLE 1

AGE IN YEARS	FSW	MSM	PROM. MEN	PROM. WOMEN	TRANSGENDER	TOTAL
18-20	1 (1.53%)	3 (10%)	1 (1.35%)	1 (4.16%)	1 (14.2%)	7 (3.5%)
21-30	26 (40%)	15 (50%)	26 (35.1%)	10 (41.6%)	4 (57.1%)	81 (40.5%)
31-40	32 (49.2%)	8 (26.6%)	31 (41.8%)	12 (50%)	2 (28.5%)	85 (42.5%)
41-50	6 (9.2%)	4 (13.3%)	16 (21.6%)	1 (4.16%)	0 (0%)	27 (13.5%)
TOTAL	65	30	74	24	7	200

The overall commonest age group in this study population is 31- 40 years.



SEX



MARITAL STATUS

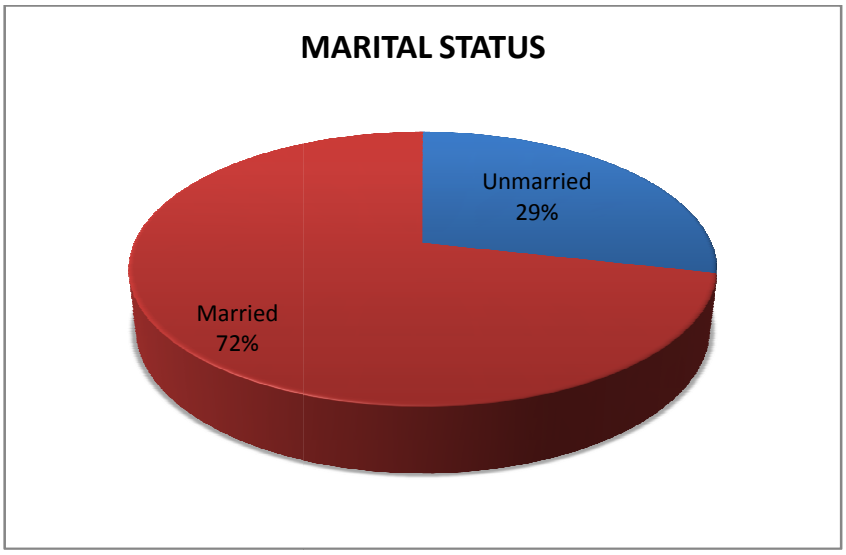
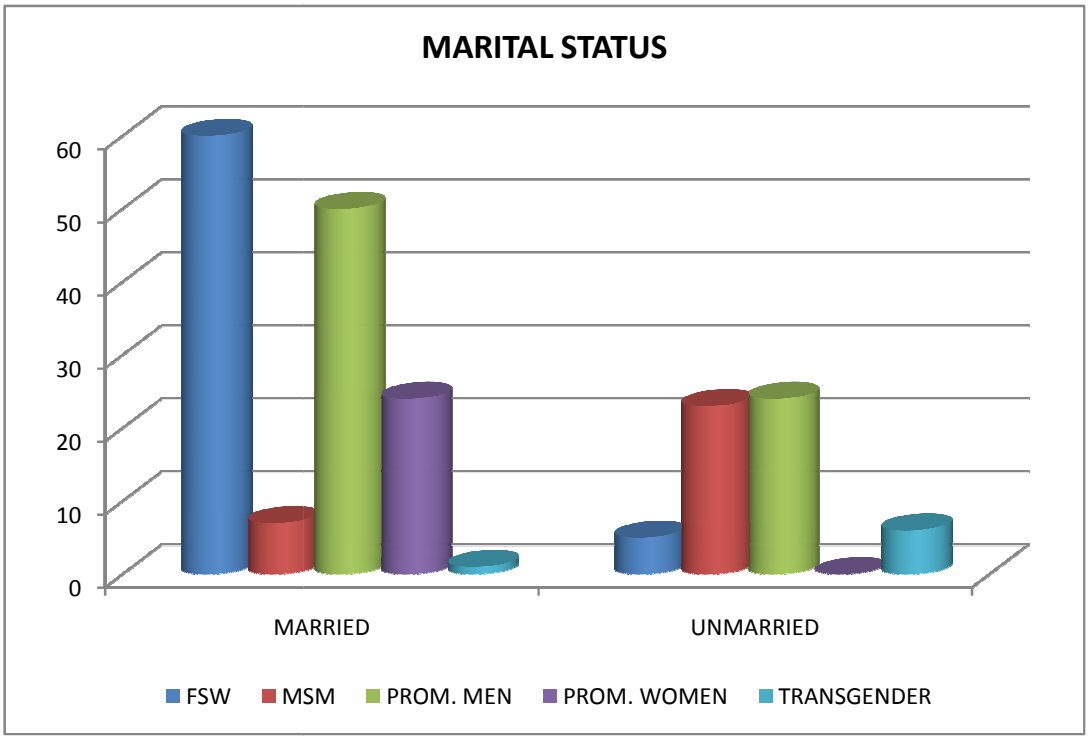


TABLE 2

MARITAL STATUS

CATEGORY	MARRIED	UNMARRIED	TOTAL
FSW	60 (92.3%)	5 (7.6%)	65
MSM	7 (23.3%)	23 (76.6%)	30
PROM. MEN	50 (67.5%)	24 (32.4%)	74
PROM. WOMEN	24 (100%)	0 (0%)	24
TRANSGENDER	1 (14.2%)	6 (85.7%)	7
TOTAL	142 (71%)	58 (29%)	200

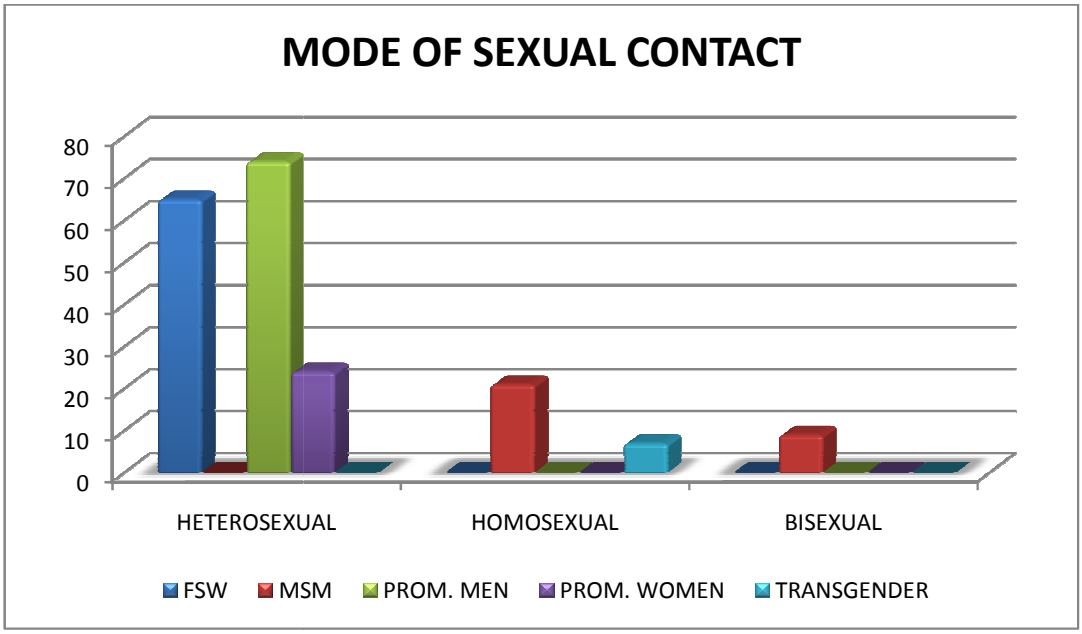
Pearson chi- square test df 4 asymptotic sigma 0.0005 (p>0.01statistically significant)



MODE OF SEX- TABLE 3

TYPES	FSW	MSM	PROM. MEN	PROM. WOMEN	TRANSGENDER	TOTAL
HETEROSEXUAL	65 (100%)	0	74 (100%)	24 (100%)	0	163 (81.5%)
HOMOSEXUAL	0	21 (70%)	0	0	7 (100%)	28 (14%)
BISEXUAL	0	9 (30%)	0	0	0	9 (4.5%)

The commonest mode of sex among FSW, Promiscuous men and women is heterosexual (81%).



CLASSIFICATION OF MALE SEX WITH MALE PATIENTS AND THEIR OBSERVED PREVALENCE

Male sex workers [MSWs] are classified into different categories based on gender, Identity, behavior and profession. Based on identity they are categorized as 'gay', bisexuals , kothis –who are ano receptive, oro receptive and both oro and ano receptive partners, Panthis- who are ano insertive, oro insertive and both ano and oro insertive and Double deckers who are both ano/oro receptive and insertive.

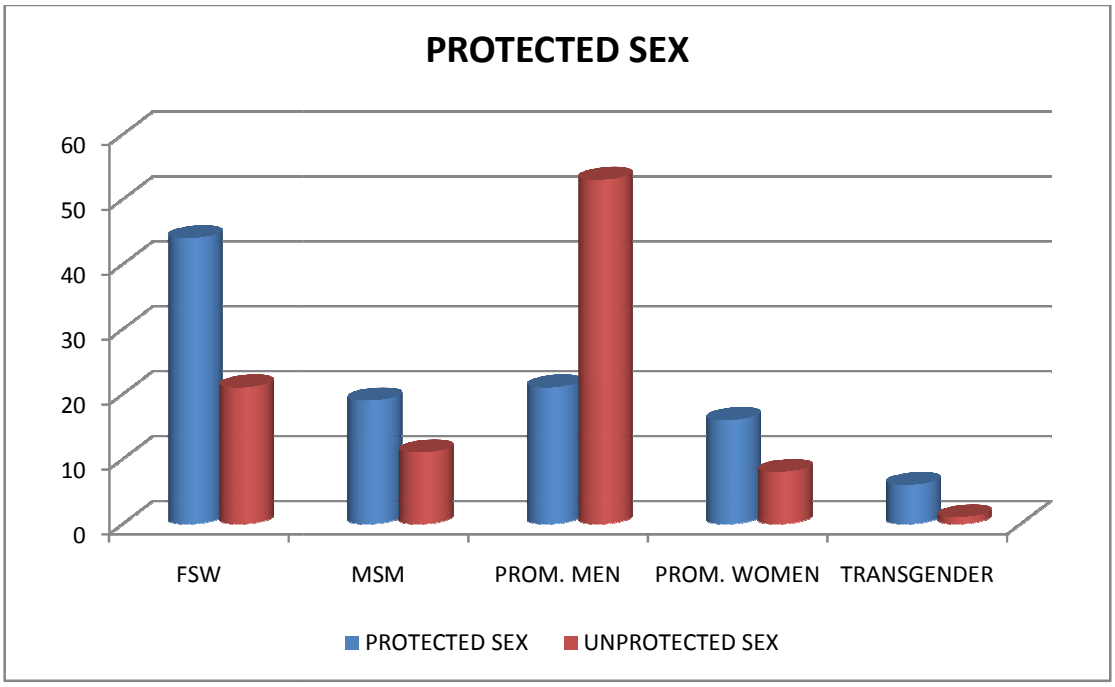
TYPE		MSM	TRANSGENDER	TOTAL
KOTHIS	**ORO RECEPTIVE (13.3%)**	**4**	**0**	**4**
	ANO RECEPTIVE (20%)	**6**	**0**	**6**
	ANO/ ORO RECEPTIVE (26.6%)	**8**	**7 (100%)**	**15**
PANTHIS	**ORO INSERTIVE (16.6%)**	**5**	**0**	**5**
	ANO INSERTIVE (6.6%)	**2**	**0**	**2**
	ORO ANO INSERTIVE (0%)	**0**	**0**	**0**
DOUBLE DECKERS	**ORO INS/ REC (0%)**	**0**	**0**	**0**
	ANO INS/ REC (3.3%)	**1**	**0**	**1**
	ORO ANO INS/ REC (13.3%)	**4**	**0**	**4**
TOTAL		**30**	**7**	**37**

Among the MSMs in our study group the commonest group is ano/oro receptive [26.6%] Kothis and among the transgenders enrolled in our study the predominant mode of sexual contact is ano/oro receptive [100%].

OBSERVATION OF PROTECTED SEX- TABLE 5

CATEGORY	PROTECTED SEX	UNPROTECTED SEX	TOTAL
FSW	44 (67.6%)	21(32.4%)	65
MSM	18 (60%)	12 (40%)	30
PROM. MEN	21(28.3%)	53 (71.7%)	74
PROM. WOMEN	16 (66.6%)	8 (33.4%)	24
TRANSGENDER	6 (85.7%)	1(14.3%)	7
TOTAL	105 (52.5%)	95 (47.5%)	200

Pearson chi- square test df 4 asymptotic sigma 0.0005 (p value <0.01 statistically significant)

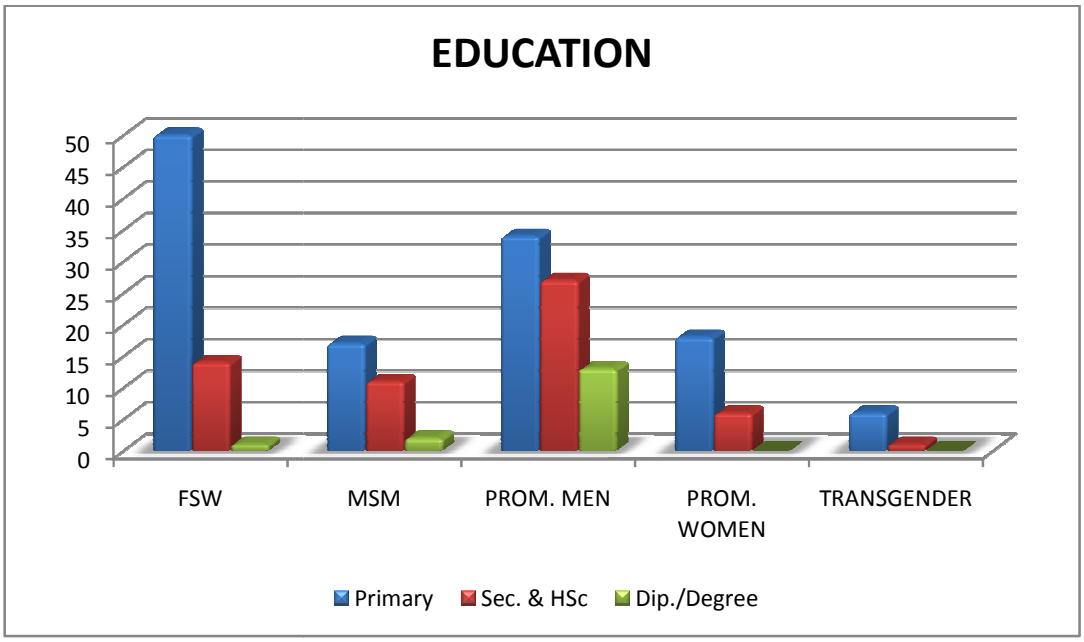


EDUCATION STATUS

TABLE 6

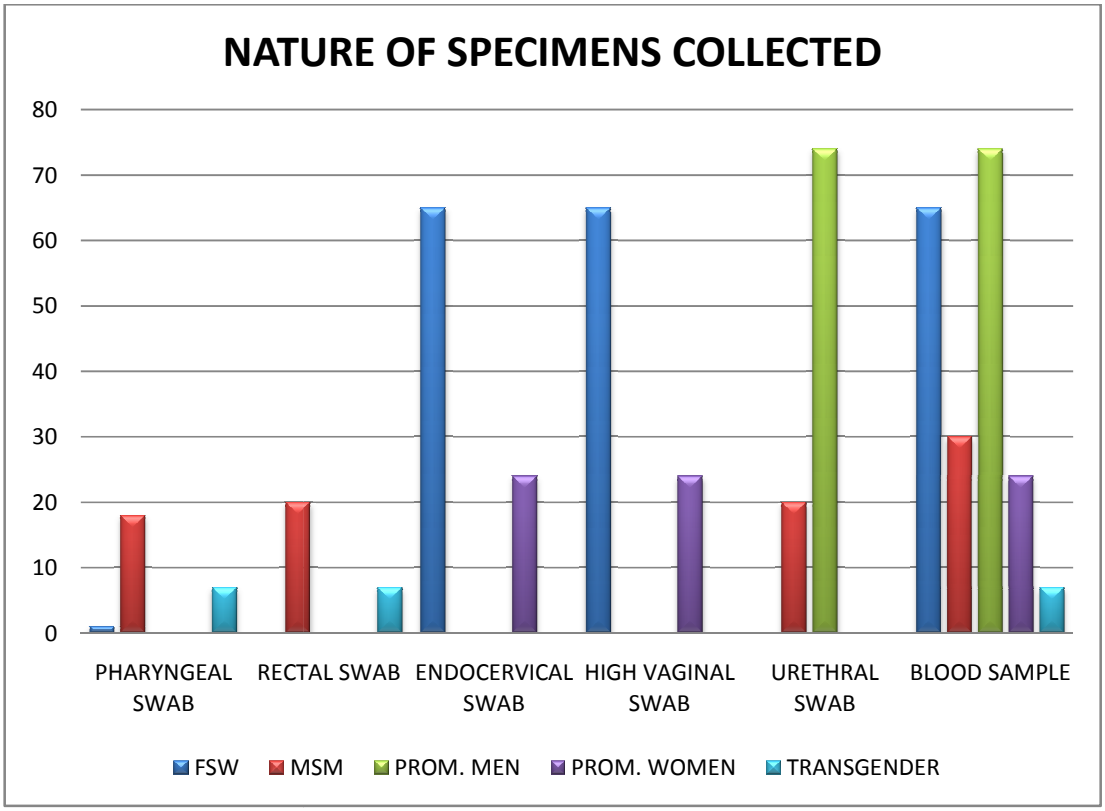
CATEGORY	PRIMARY SCHOOL (UPTO 8TH GRADE)	SEC. AND HIGHER SEC. SCHOOL(9th to 12th GRADE)	COLLEGE (DIPLOMA/DEGREE)
FSW	50 (76.9%)	14 (21.5%)	1 (1.5%)
MSM	17 (56.6%)	11 (36.6%)	2 (6.6%)
PROM. MEN	34 (45.9%)	27 (36.4%)	13 (17.5%)
PROM. WOMEN	18 (75%)	6 (25%)	0 (0%)
TRANSGENDER	6 (85.7%)	1 (14.3%)	0 (0%)
TOTAL	125 (62.5%)	59 (29.5%)	16 (8%)

The educational status of the subjects in high risk group in predominantly primary level (62.5%).



NATURE OF SPECIMENS COLLECTED- TABLE 7

Category	Pharyngeal swab	Rectal swab	Endocervical swab	High vaginal swab	Urethral swab	Blood sample
Fsw	1	0	65	65	0	65
Msm	18	20	0	0	20	30
Prom. Men	0	0	0	0	74	74
Prom. Women	0	0	24	24	0	24
Transgender	7	7	0	0	0	7
Total	26	27	89	89	94	200



High vaginal and endocervical swabs were collected from all the 65 female sex workers. Rectal and Pharyngeal swabs were collected from all 7 transgenders. Urethral swabs were collected from all the 74 promiscuous men and pharyngeal swab was collected from 10 promiscuous men and rectal swab was collected from 1 promiscuous man. Endocervical and high vaginal swabs were collected from all 24 promiscuous women. One pharyngeal swab was collected from a female sex worker who admitted of practicing oral sex. Blood was collected from all 200 patients for doing serological tests

OTHER SEXUALLY TRANSMITTED ILLNESS FOUND IN THE STUDY POPULATION

POSITIVITY IN DIRECT EXAMINATION- TABLE 8

Tests Done	Positive Findings	High vaginal swab (89)	Endocervical swab (89)	Urethral swab (94)	Rectal swab (27)	Pharyngeal swab (26)	Total
Wet Mount (89)	Clue cells	12	0	0	0	0	12
	Yeast cells with and without budding mycelium	11	0	0	0	2	13
	Motile trichomonads	3	0	0	0	0	3
10% KOH mount	Yeast cells with / without budding mycelium	11	0	0	0	2	13
	Fishy odour/ amine test	12	0	0	0	0	12
Grams stain (325)	Epithelial cells with lactobacillus	59	0	0	0	0	59
	Clue cells	12	0	0	0	0	12
Nugent score 7- 10	Lactobacillus 1-4, <1	12					12
	Mobiluncus >5, 1-4	12					12
	Gardrenella 5-30, >30	12					12
Pus cells and intracellular gram neg. diplococcic				3	2		5
Yeast cells with/ without budding mycelium		11				2	13

Interpretation

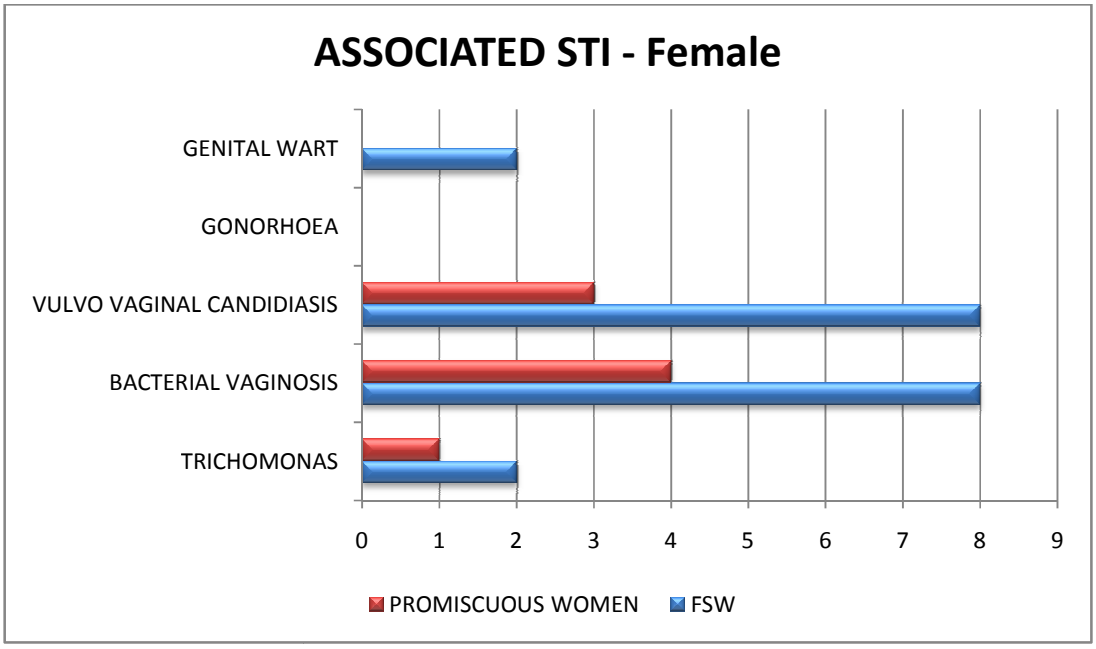
Motile, treponemes under dark ground illumination were observed in all 10 cases of hard chancre. Multinucleated giant cells with faceted nuclei and homogenously stained ground glass chromatin (Tzanck cells) were observed in both cases of herpetic ulcers.

Among the high vaginal swabs collected in FSW and promiscuous women, the wet mount showed clue cells in ^{12}/89 (13.4%) which is in accordance with the gram stain. Yeast cells with and without budding mycelium was seen in 13/200 (6.5%) which is in accordance with 10% KOH mount and motile trichomonads were seen in 3/ 89 (3.37%) , Gram stain of the specimen collected showed pus cells and intracellular gram negative diplococci in 5/200 (2.5%). The gram stain showed clue cells in 12/89 (13.4%) and Nugent[16,20] score of 7 to 10 was seen in all 12 subjects 12/12 (100%).

ASSOCIATED STI BY DIRECT EXAMINATION IN THE FEMALE POPULATION IN OUR STUDY GROUP (FSW AND PROMISCUOUS WOMEN)

TABLE 9

Category	Trichomonas	Bacterial vaginosis	Vulvo vaginal candidiasis	Gonorrhoea	Genital wart
Fsw (65)	2	8	8	0	2
Promiscuous women (24)	1	4	3	0	0
89	3	12	11	0	2

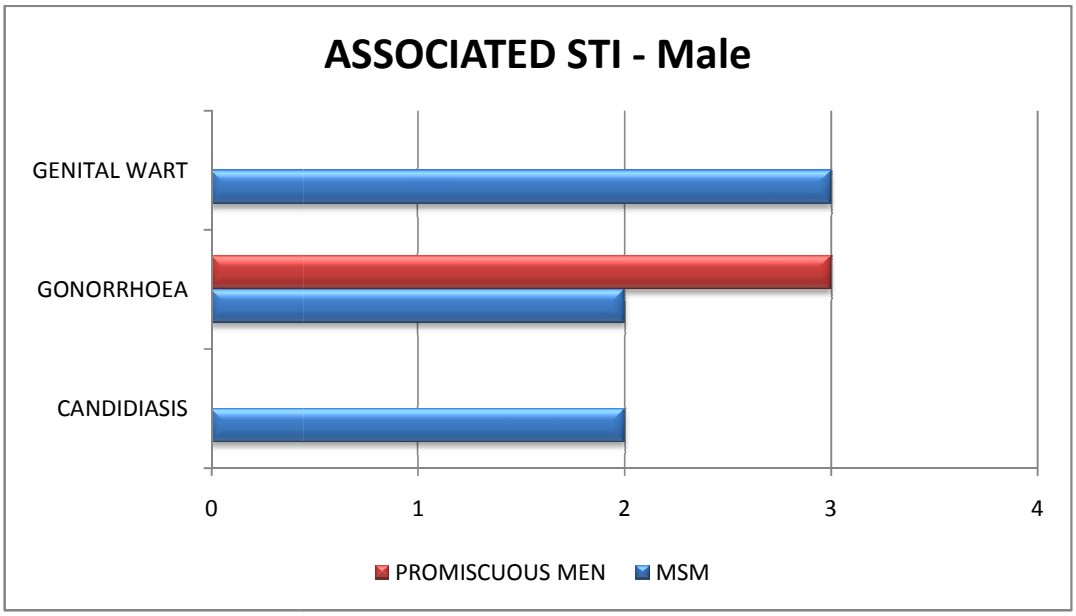


ASSOCIATED STI BY DIRECT EXAMINATION FOUND IN THE MALE POPULATION IN OUR STUDY GROUP (MEN HAVING SEX WITH MEN AND PROMISCUOUS MEN)

TABLE 10

CATEGORY	CANDIDIASIS	GONORRHOEA	GENITAL WART
MSM (30)	2	2	3
PROMISCUOUS MEN (74)	0	3	0
104	2	5	3

No associated STI was identified in the transgender population in the direct method.



GENITAL ULCER SYNDROME

TABLE 11

CATEGORY	HERPETIC	NON HERPETIC	
		SYPHILITIC	OTHERS
FSW (65)	0	1	0
MSM (30)	1	1	0
PROMISCUOUS MEN (74)	1	6	0
PROMISCUOUS WOMEN (24)	0	2	0
TRANSGENDERS (7)	0	0	0
TOTAL (200)	2	10	0

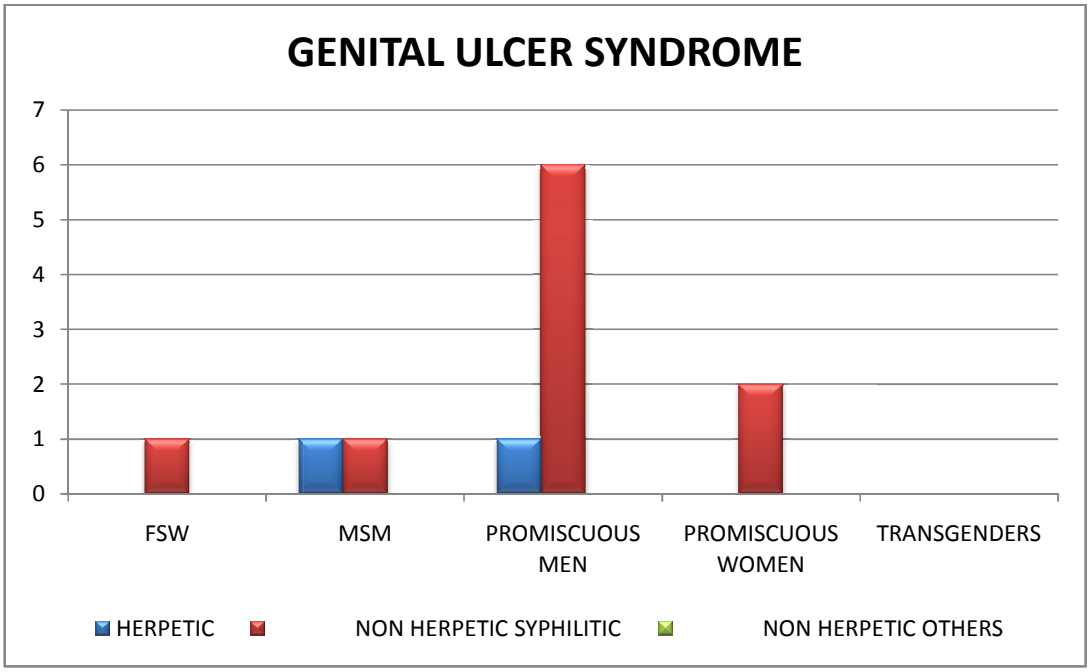
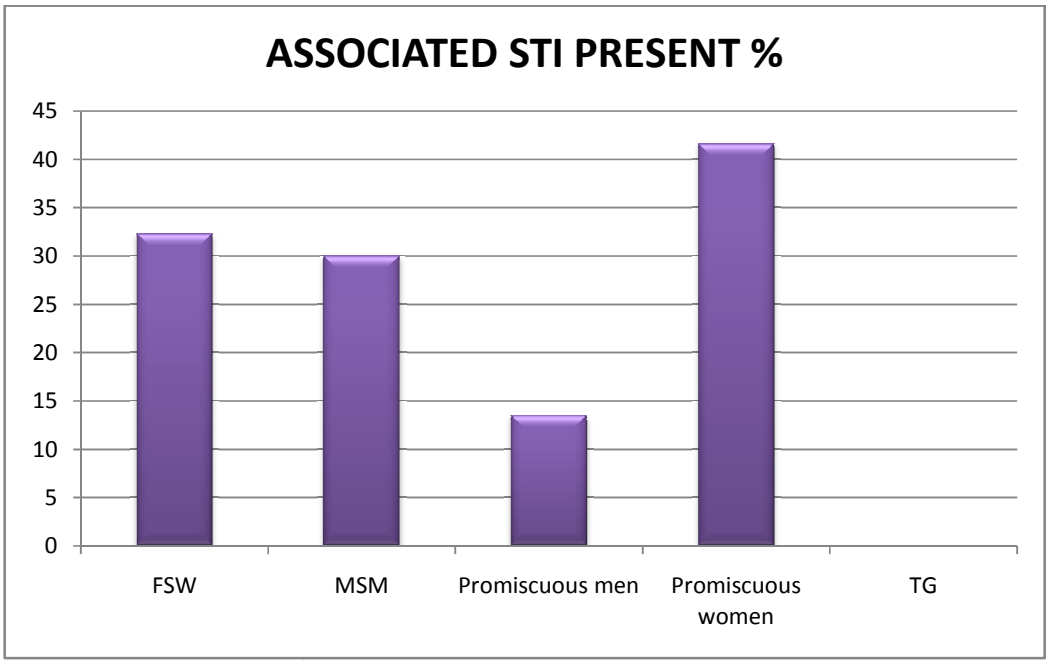


TABLE 12

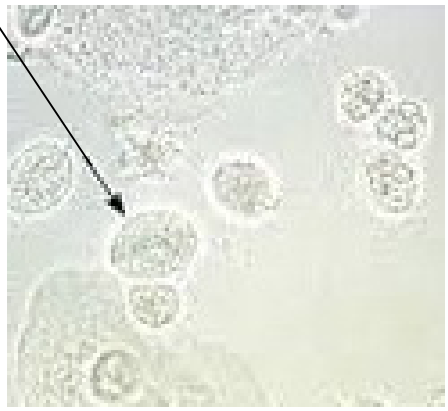
CATEGORY	ASSOCIATED STI PRESENT IN
FSW - 65 subjects	21 subjects (32.3%)
MSM – 30 subjects	9 subjects (30%)
Promiscuous men- 74 subjects	10 subjects (13.5%)
Promiscuous women- 24 subjects	10 subjects (41.6%)
Transgenders- 7 subjects	0 (0%)

Pearson chi- square test df 4 asymptotic sigma 0.01 (p value<0.05statistically significant)

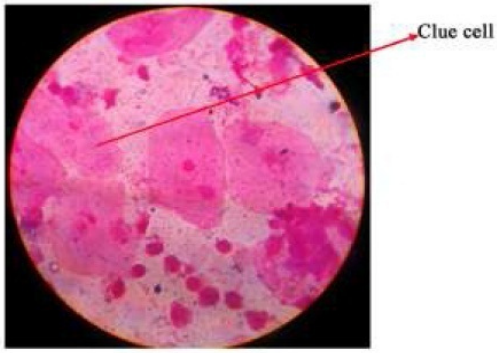


TRICHOMONAS VAGINALIS WET MOUNT

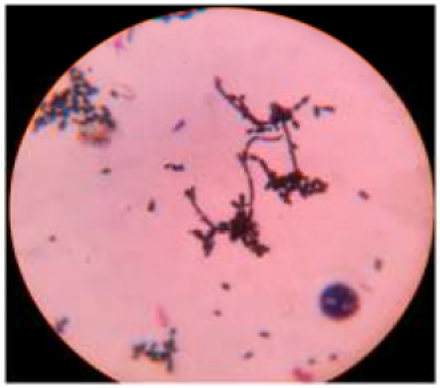
T. VAGINALIS



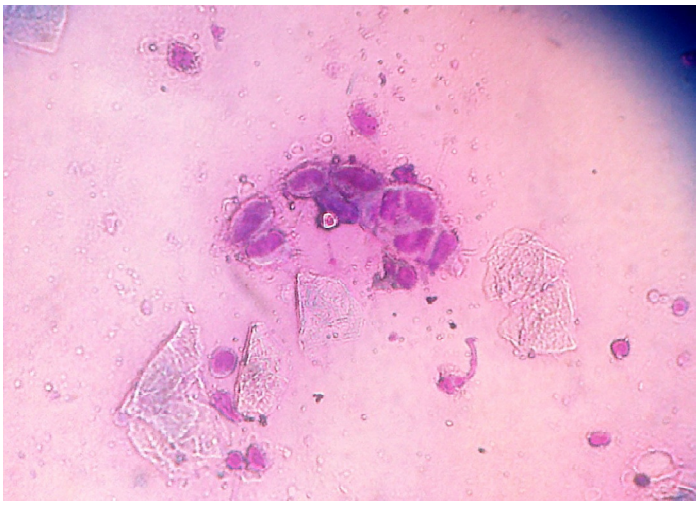
BACTERIAL VAGINOSIS- GRAM STAIN



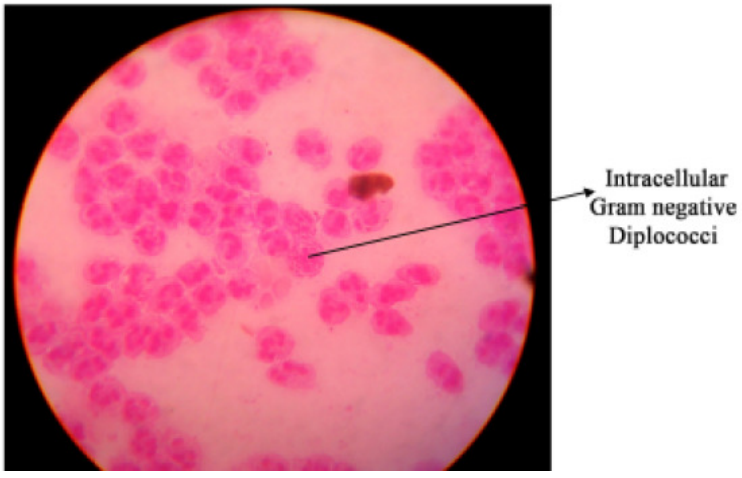
CANDIDA - BUDDING YEAST CELLS WITH PSEUDO HYPHAE



HERPES GENITALIS - TZANCK SMEAR OBSERVATION OF MULTINUCLEATED GIANT CELLS



GONOCOCCI - URETHRAL SMEAR GRAM STAIN

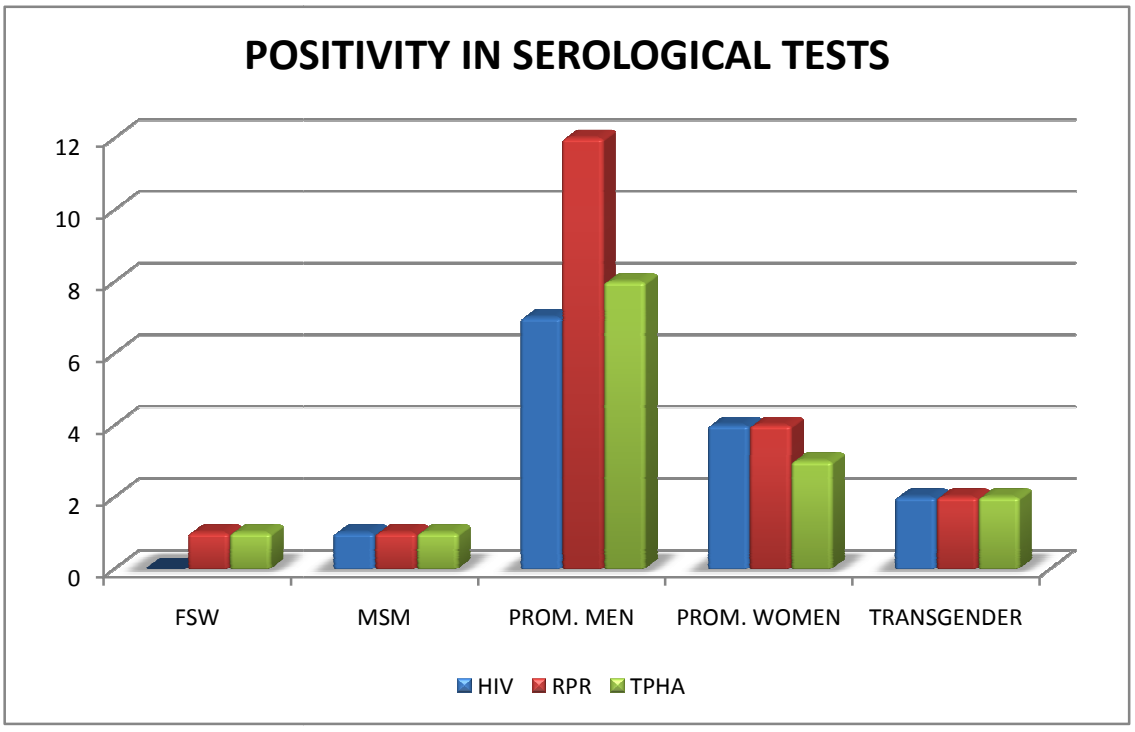


POSITIVITY IN SEROLOGICAL TESTS

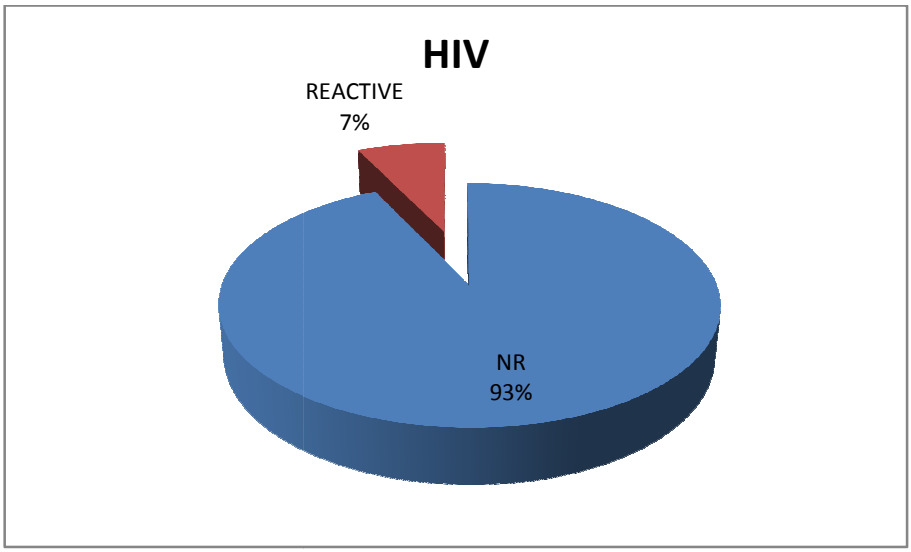
TABLE 13

TEST DONE	FSW	MSM	PROM. MEN	PROM. WOMEN	TRANSGENDER	TOTAL
HIV	0	1	7	4	2	14
RPR	1	1	12	4	2	20
TPHA	1	1	8	3	2	15

RPR- Pearson chi- square test df 4 asymptotic sigma 0.008 (p value <0.05 statistically significant)



HIV- Pearson chi- square test df 4 asymptotic sigma 0.005 (p value <0.05 statistically significant)

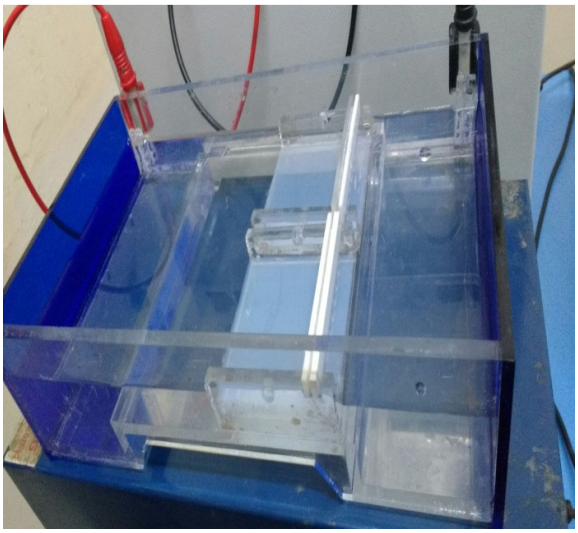


PREVALENCE OF HPV 16 & 18

HPV PCR test was conducted on the appropriate samples of all the 200 patients and the results were obtained as follows-

THERMOCYCLER

GEL ELECTROPHORESIS



MULTIPLEX PCR FOR HPV 16 AND 18

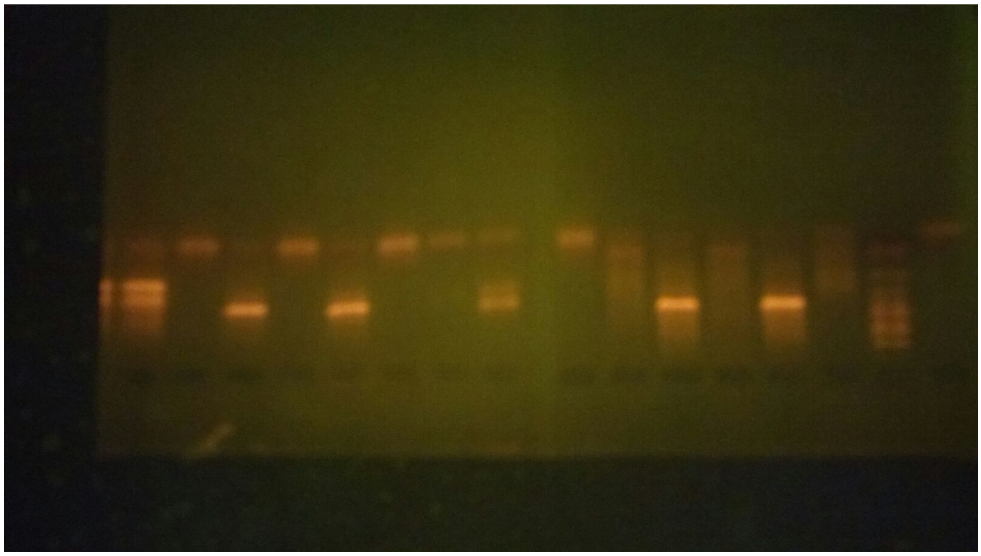
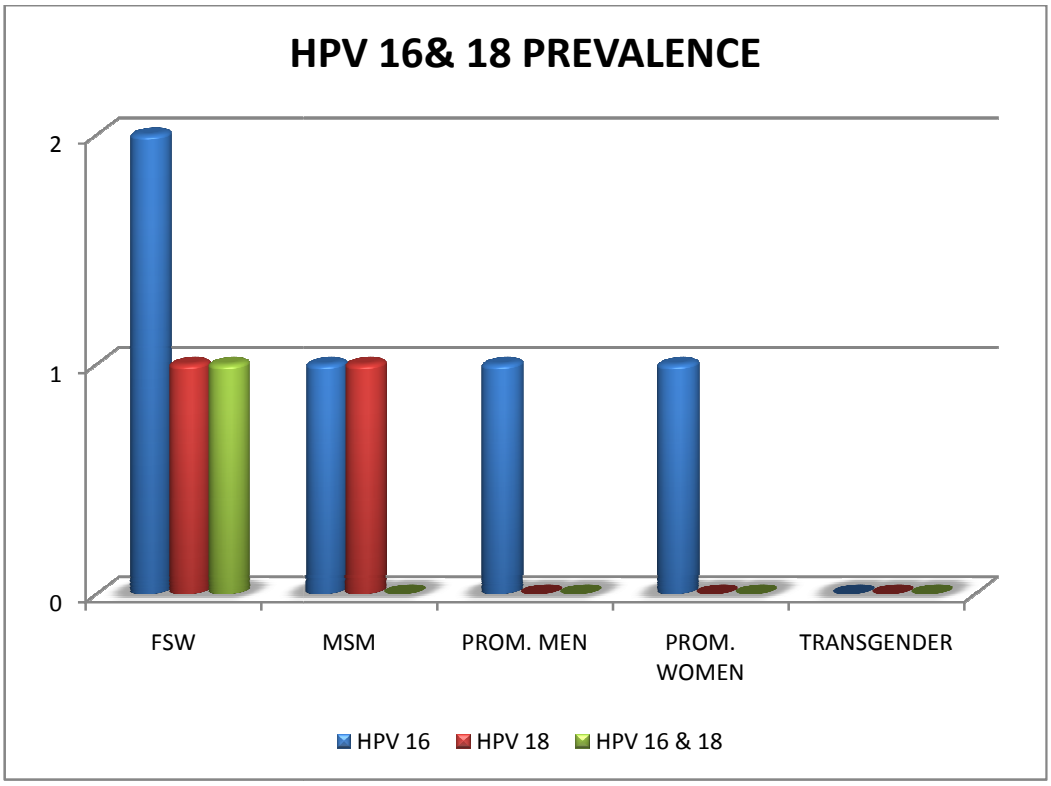


TABLE 14- PREVALENCE OF HPV 16 AND 18 BY PCR

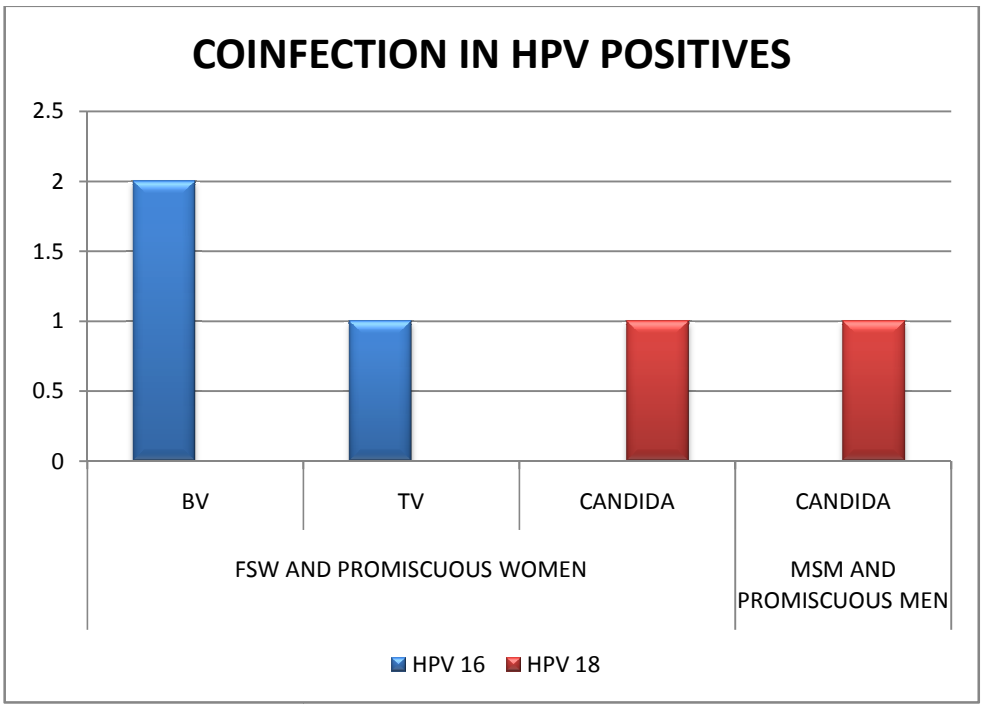
CATEGORY	HPV 16	HPV 18	HPV 16 & 18
FSW	2	1	1
MSM	1 (in kothis)	1 (in kothis)	0
PROM. MEN	1	0	0
PROM. WOMEN	1	0	0
TRANSGENDER	0	0	0
TOTAL	5	2	1



COINFECTION IN HPV POSITIVES

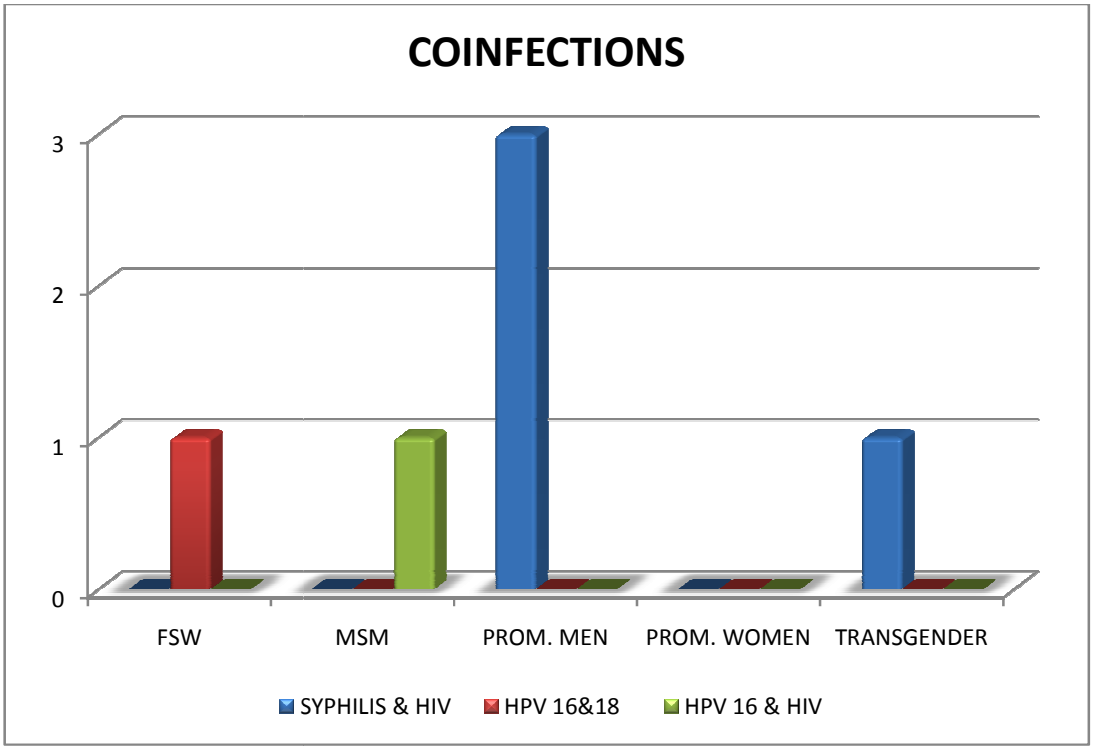
TABLE 15- COINFECTION IN HPV POSITIVE SUBJECTS

HPV type	**FSW AND PROMISCUOUS WOMEN**			**MSM AND PROMISCUOUS MEN**	
	BV	**TV**	**CANDIDA**	**CANDIDA**	**GONORRHOEA**
HPV 16	2	1			
HPV 18			1	1	
HPV 16 AND 18					



COINFECTION OF SYPHILIS, HIV AND HPV- TABLE 16

Category	FSW	MSM	Promiscuous men	Promiscuous women	Trans Gender	Total
SYPHILIS & HIV	0	0	3	0	1	4
HPV 16&18	1	0	0	0	0	1
HPV 16 & HIV	0	1	0	0	0	1



ASSOCIATION OF BLOOD TRANSFUSION AND IV DRUG ABUSE

Blood transfusion history was present in a female sex worker and a promiscuous man. Intravenous drug abuse was present in 4 promiscuous men. All were HPV PCR negative.

TABLE 17

CATEGORY	BLOOD TRANSFUSION	IV DRUG ABUSE
FSW	1	0
MSM	0	0
PROM. MEN	1	4
PROM. WOMEN	0	0
TRANSGENDER	0	0
TOTAL	2 (0 HPV PCR)	4 (0 HPV PCR)

DISCUSSION

The prevalence of Human papilloma virus in our hospital was analysed in high risk groups in 200 subjects.

The age group patterns are shown in Table 1. The commonest age observed in female sex workers is 31-40 years which is in concordance with the study of Pallavi Shukla et al[22]. In our study the commonest age group among men having sex with men and transgenders was 21 to 30 years which is in concordance with studies of Abarna Devi et al[23] and Dharma Nand Bhatta[24]. The commonest age group among promiscuous men and women who constituted the majority of the study population is 31 to 40 years which is in concordance with the studies conducted by Susan Walsh[25]. The overall commonest age group in our study population is 31 to 40 years.

The Marital status among the study population is shown in Table 2. Majority of Female sex workers (92.3%) in our study group were married which is in concordance with studies of Hemalatha et al[26]. Men having sex with men and transgenders in our study group were mostly unmarried (76.6% and 85.7%) which is in concordance with studies of HuyHa et [al 27].Promiscuous men and women in our study group were married (67.5% and 100% respectively) which is in concordance with studies of Shilpi Choudhry et al[28].

The mode of sex (shown in Table 3) among female sex workers, Promiscuous men and women in our study group is Heterosexual mode which is in concordance with the studies of Abarna Devi et al.[23] The mode of contact

among men having sex with men(70%) and transgenders(100%) is homosexual while only 30% of men having sex with men were bisexual. This finding also coincides with the finding of Abarna Devi et al[23].

Among men having sex with men, the predominant mode of contact is ano/ oro receptive (kothis 26.6%) shown in Table 4. The sexual mode among transgenders is also ano/ oro receptive (100%). The above finding is in concordance with studies of Tanmay Mahapatra et al[29].

The last sexual contact (Table 5) among female sex workers, men having sex with men, Promiscuous men and women and transgenders was protected. This can be explained by the increasing female literacy rate, improved health care seeking behavior and awareness among the high risk groups[30]. The last sexual contact was predominantly unprotected (71%) among promiscuous men which is in concordance with the studies of Abarna Devi et al[23]. This is of health care significance as it constitutes a major reason for spread of sexually transmitted diseases. Health care providers must be taught of the significance of this issue with screening programmes to detect STI in this group.

The educational status is shown in Table 6. 62% of our study population had completed only primary school education whereas 29% had completed secondary and higher secondary school education. Only 8% were diploma/degree holders. This observation was in concordance with studies of Solomon MM et al[31].Commercial sex workers with lower educational level

were afflicted more with sexually transmitted infections and participated in high risk behaviours. Majority of the female sex workers were among the primary school level in the study conducted by Melissa Sohoo et al[2].

The types of specimens collected in the study group is shown in table 7. As the mode of sexual contact among female sex workers and promiscuous women was heterosexual, high vaginal and endocervical swabs were taken from all the 65 female sex workers and 24 promiscuous women. As only one among the female sex workers admitted of practicing oral sex, a pharyngeal swab was also taken from her in addition. As the mode of contact among promiscuous men was heterosexual, urethral swabs were taken from them. Among the Male sex with male patients, swabs were taken according to their sexual habits. Rectal swabs for ano receptive, pharyngeal swabs for oro receptive and both from ano/ oro receptives were taken. Urethral swabs were taken for oro/ ano insertive MSM and all three swabs were taken from double deckers(ano/oro receptive/insertives). 7 out of 30 MSMs in our group reported bisexual mode of contact and hence urethral swabs were also taken from them in addition. The mode of contact among transgenders was ano/ oro receptive and hence rectal and pharyngeal swabs were taken from all 7 transgenders practicing commercial sex. Blood was collected from all 200 subjects for performing serological tests for HIV and Syphilis (RPR).

The positivity in direct examination and the concominant STI in the male and female population in the high risk groups in our study population is shown in tables 8 to12. Associated sexually transmitted infections were found

in 50 subjects (25%) of which 21 FSWs, 9 MSMs, 10 promiscuous men and 10 promiscuous women. Positivity in direct examination was observed for Trichomoniasis, Bacterial Vaginosis, Candidiasis and Gonorrhoea. The prevalence of Trichomoniasis in our study group is 3.37% which is slightly higher when compared to the studies of Nimisha D Shethwala et al[32] which is only 2%. The prevalence of Bacterial vaginosis in our study group was 13.4% which is in concordance with the study of Nimisha D Shethwala et al[32] which was 13.3%.The prevalence of candidiasis in our study is 12.3% which is slightly higher than the study of Nimisha D Shethwala et al[32] which is 10.3%.The prevalence of Gonorrhoea in our study group was 4% which is less when compared to the study of Vandana et al[33] which is 11%.

Among the types of genital ulcers, there were 2 cases (1%) of herpetic ulcers and 10 cases (5%) of syphilitic ulcers. There were no other types of ulcers found. Genital warts was found in 5 cases (2.5%). As genital warts are caused by serotypes 6 and 11, these patients were also included for evaluating high risk HPV 16 and 18.

Positivity in serological tests among the high risk groups is shown in table13. HIV was reactive among 7% of our study population. The prevalence of HIV was 0% among FSW, 3.3% among MSM. According to recent NACO statement, HIV prevalence among FSW and MSM are 2.67% and 4.43% respectively[35]. According to NACO, the prevalence of HIV among transgenders is between 17.5% to 41%[35]. In our study the HIV prevalence among transgenders is 28.5%. HIV prevalence was 10.5% among promiscuous

men and 12.5% among promiscuous women among our study. Rapid plasma reagin test (RPR) was reactive/ positive in for 1.5% in FSW, 3.3% in MSM and 28.5% in transgenders. Though the RPR was reactive in 20 subjects, TPHA was positive only in 15 subjects. This could be due to biological false positivity observed in RPR. The percentage among MSM in Pisani et al[36] studies is 2%. The prevalence of syphilis in transgender is 13.6% in Brahman et al[37] studies. The prevalence of syphilis among FSW in our study is low (1.5%) when compared to the studies of Uribe Salas (8.2%)[38]

The prevalence of HPV 16 and 18 infection by PCR is shown in table 14. HPV infection is mainly diagnosed by molecular methods since reliable serological tools are not available and culture of the virus is not possible. Since HPV cannot be propagated in tissue culture its accurate identification relies on molecular biology techniques. The PCR based techniques are highly sensitive, specific, reliable diagnostic tool for detecting targeted HPVs in tissue and cellular samples. Worldwide the most common HR-HPV (High risk- Human papilloma virus) are HPV 16/18 serotypes and are implicated in 70% of cervical cancers and 70% of anal cancers in men and 40-60 % of vulvar ,vaginal and penile cancers. [21.] Hence type specific PCR to detect HPV 16/18 serotypes was performed.

Approximately 291 million women worldwide are HPV DNA carriers. 32% of the HPV infected women from the general population are infected with High risk types 16 and 18[2]. The median prevalence of high risk HPV by region was Africa (8%), America (11.1%), Eastern Mediterranean (13.7%), Europe

(11.5%), South East Asia (13.9%), Western Pacific (6.9%). The prevalence of HPV type 16 ranged from 1.1% to 38.9% across all regions[2]. The most prevalent type reported among female sex workers included HPV 16 (38.9%) and HPV 18 (23.1%)[2]. In east Asia the most prevalent genotypes were HPV 16 (23.9%) and HPV 18 (11%).

In our study, the overall high risk HPV prevalence was 4% which is very low. HPV 16 was found in 2.5%, HPV 18 in 1% and HPV 16&18 in 0.5%. The prevalence was among 4 female sex workers aged 20 to 30 years, 2 MSMs aged 20 to 30 years, 1 promiscuous man aged 20 to 30 years and 1 promiscuous woman aged 20 to 30 years. HPV prevalence is high among younger age groups with increased sexual activity. The prevalence of HPV infection decreases with increasing age due to the clearance of infection and natural immunity and constant production of HPV systemic antibodies which contributes to an immune response against new infection[4].

FSWs have a 10 fold increased risk of HPV infection than the general population in the study by Peng RR et al[39]. In this study, a meta analytic approach to systematically analyse the literature to elucidate the prevalence and genotype distribution of cervical HPV infection among FSWs in Asia was done which showed a overall prevalence among FSWs to be 12.8% to 84.8%. The most prevalent genotypes were HPV 16(23.9%) and 18 (11%) in East Asia and HPV 52 (12.9%) and HPV 16 (8.5%) in south east Asia. High risk HPV prevalence was 28% in Spaniards, 32% in Latin Americans and Eastern Europeans. The prevalence was 16% in sub-Saharan Africans and 16% in north

Africans. High risk HPV 16 was the most common type detected 24% and HPV 18 was 11%[40]. In another study, overall HPV prevalence among FSW was 38.7% with high risk HPV 26.5% and HPV 16 was 8.3%[41].

Studies have shown that having multiple sex partners may lead to higher HPV transmission. Hence female sex workers are at greater risk of infection when compared to the general population. Moreover HPV can be transmitted from FSW to the general population through clients thereby increasing the prevalence of the virus[2]. In our study, HPV prevalence among the FSWs was (4/65) i.e., 6.2%. This is low compared to results of many studies. The reason for a low HPV in FSW could be due to a 67.6% protected sex found in our study in this group.

In our study the prevalence of HPV among MSM was 6.6% (HPV 16 3.3% and HPV 18 3.3%). 1 MSM who was positive for HPV 16 was also HIV reactive. Anal canal is the common site in this group and anal cancer incidence is estimated to be 44 times higher than the general population. Among the MSM, ano receptive intercourse is more prone for HPV infection. HIV infected MSM are at particularly high risk for anal HPV infection. Studies show that upto 95% HIV infected MSM also harbor anal HPV infection and their risk of anal cancer is 60 times higher than that of general population[42].

HIV infection is a strong and independent determinant for HPV seropositivity due to the persistence of HPV infection in HIV infected MSM. Seroprevalence of high risk HPV 16 among HIV negative MSM was 37.1%

whereas in HIV positive MSM it was 62.7%. Similarly sero prevalence of HPV 18 was also high in MSM who were HIV positive (42.5%) when compared to MSM who were HIV negative (29.1%)[43]. In various studies conducted to detect the prevalence of HPV 16 and 18 in MSM the results were HPV 16 17%, HPV 18 (32.5%)[44]. HPV 16 prevalence was 24.5%. In another study by Maria A Pando[45], HPV 16 was the most common type detected among MSM and MSM & W and MSW(Male Sex Worker) and is responsible for most HPV associated penile cancers[46]. Penile high risk HPV prevalence was 16% in HIV negative and 32% in HIV positive MSM. Prevalence of anal high risk HPV prevalence was 45% in HIV negative and 65% in HIV positive MSM. HPV 16 was detected in 22% of HIV positive MSM and 13% of HIV negative MSM[47]. Prevalence of anal canal HPV 16 was more than double among MSM when compared to heterosexual men (6.3% and 2.2%)[48]. HPV 16 and 18 seropositivity was high in ano receptive MSM when compared to insertive MSM[47]. In our study the prevalence is low (6.6%) when compared to many studies. In our study there was a 60% protected sex rate. This could have been the reason for low prevalence found in our study.

Among the transgender population in our group, HPV 16 or 18 was not detected in any of the 7 subjects in our study. In a study conducted by Maria Sol Dos et al, the prevalence of HPV 16 and 18 was found to be 82.5% among transgenders.[49].

HPV 16 was identified in 1 out of 74 promiscuous men and 1 out of 24 promiscuous women. Promiscuity is the practice of having casual sex

frequently with different partners and being indiscriminate in the choice of sexual partners. In India sexual promiscuity is considered immoral, sinful and is a deviation from the acceptable moral standards. Along with modernization, attitude regarding sex are changing fast and promiscuity is on the increase indicating the weakening of the traditional familial and societal controls on sexual behavior[50]. Although there are no conclusive studies to show that promiscuous men and women have high HPV prevalence, it is important to screen this group as we found that it is the group with the highest incidence of unprotected sex.

. Coinfection of concomitant sexually transmitted infections in HPV 16 and 18 positive subjects is shown in table 15. In our study population, 2 female sex workers positive for HPV-16 by PCR also had associated Bacterial vaginosis, and one female sex worker who was positive for HPV-16 also had associated Trichomoniasis. One Promiscuous male and one promiscuous female with HPV 18 positivity had associated Candidiasis. This finding is in accordance with the observation of C.Rodriguez et al[8]. Bacterial vaginosis associated organisms produce cytokines, inflammatory mediators and enzyme sialidases. The enzymes destroy protective mucosal barriers and increase susceptibility to cervical HR-HPV infection by facilitating adherence, invasion and eventually incorporation of HPV activated oncogenes in to the genome of cells in the transformation zone. Abnormal vaginal microbiota may also be implicated in the maintenance of subclinical HR-HPV infection. Enzymes that are produced by anaerobic bacteria and are involved in the pathogenesis of

Bacterial vaginosis can potentially alter immune signals and promote degradation of protective factors rendering women more susceptible to acquiring HR-HPV. The double stranded RNA of T.Vaginalis is associated with differential expression of enzymes which affect virulence and alter the history of various STI in particular HR-HPV. Hence patients with Bacterial vaginosis and Trichomoniasis is at significant risk of acquiring High risk HPV[8].

Coinfection of syphilis and HIV and HPV is shown in table 16. In our study the prevalence of coinfection between HIV and Syphilis among the high risk groups was 2% which is slightly higher than the prevalence observed in the studies of Yan Luo et al[51] which is 1.5% and slightly lower than the prevalence observed in the study of Aritra Das et al[52]which is 2.6%. Coinfection of HIV and HPV in our study group is 0.5% among the 200 subjects (compared to the studies of Lei Gao et al[53] which is 8.3%) but the percentage of HPV positivity in HIV positive MSM was 100%. The percentage of HPV positivity in HIV positive MSM was 65% in the studies of Alberts CJ et al[54] and 62.7% in the studies of Mooji SH et al.[47]

History of blood transfusion was found in 2 subjects but they were not positive for any serological test and they did not have any associated illness (table 17). Intravenous drug abuse history is correlated with increased HPV infection due to high risk behaviour[55]. In our study IV drug abuse was present in 4 promiscuous men but were negative for HPV PCR.

SUMMARY

- Out of 200 subjects in this prospective study, Promiscuous men and Female sex workers form the majority.
- The common age group was 31- 40 years
- Most of the subjects were married (71%) except MSM and Transgender population.
- The mode of sex among the study group was heterosexual (81.5%).
- Among the men having sex with men, the common type of sex was oro ano receptive (26.6%) kothis, followed by ano receptive (20%).
- The last sexual contact was protected in 52.5% of the subjects.
- 62.5% of the subjects had only primary level of education.
- Concomitant sexually transmitted illnesses were present in 25% (50 out of 200) subjects.
- Candidiasis was found in13, Bacterial vaginosis in 12, Syphilitic ulcer in 10, Gonorrhoea in 5, Genital warts in 5 and Trichomoniasis in 3 and Herpetic ulcer in 2 of the 50 subjects positive for other STIs.
- In serological evaluation, HIV was reactive in 14, RPR in 20 and TPHA in 15 subjects.
- HPV 16 and 18 was positive in 8 subjects by PCR. HPV 16 was positive in 5, HPV 18 in 2 and HPV 16 and 18 in 1 subject.

- Coinfection in HPV positive subjects with other STIs was present in 5 subjects (Bacterial vaginosis in 2, Candidiasis in2 and Trichomoniasis in 1 out of the 8 HPV positives)

- Coinfection of HPV 16 and 18 was observed in 1 subject in1, HPV 16 and HIV in 1 and HIV and syphilis in 4 subjects.

- Blood transfusion and IV drug abuse was observed in 2 and 4 subjects respectively. No HPV positives were found in this group.

CONCLUSION

- The study on the prevalence of Human Papilloma Virus in high risk group subjects was done in a total of 200 subjects comprising 65 female sex workers, 30 men having sex with men, 74 promiscuous men, 24 promiscuous women and 7 transgenders attending STD clinic in our institution between September 2014 to September 2015.

- As the presence of concomitant sexually transmitted infections increase the risk of acquisition of HR- HPV 16 and 18, they were also identified as a part of the study.

- The HPV 16 & 18 PCR analysis of 200 anogenital and pharyngeal swabs detected 8 HPV positives which included 5 HPV16, 2 HPV18 and 1 HPV 16 & 18.

- Candidiasis was found in 13(26%), Bacterial vaginosis in 12 (24%), Syphilitic ulcer in 10 (20%), Gonorrhoea in 5 (10%), Genital warts in 5(10%) and Trichomoniasis in 3 (6%), Herpetic ulcer in 2 (4%) of the 50 positive for other STIs.

- High risk HPV 16 and 18 serotypes are associated with anogenital and head and neck cancers. The high risk group can have asymptomatic infection and can transmit the infection to the general population through their clients. Hence regular screening of this high risk groups for HPV is essential.

- HPV vaccine which targets HPV 16 and 18, the most common oncogenic types, is recommended to protect this high risk group from aggressive cancers of the anogenital region.
- HPV vaccine induces a protective host immune response which is stronger and long lasting and includes partial cross protection against non vaccine related serotypes.
- Education about safer sexual practices, regular screening for Sexually transmitted infections and prophylactic HPV vaccination in this high risk groups will protect the individuals and also prevent the transmission of infection to the general population..

BIBLIOGRAPHY

1. *Deepika Pandhi, Sidharth Sonthalia*- Human papilloma virus vaccines: currect scenario *IJ STD and AIDS* 2011/ Vol 32/ issue 2 page 75-85
2. *Melissa Soohoo, Magaly Blas, Gita Byraiah, Cesar Carcamo, Brandon Brown*

   Cervical HPV infection in female sex workers: a global perspective, *open aids journal* 2013
3. *Juarez- Figueroa, Luis A, Wheeler*- HPV a highly prevalent STD agent among female sex workers from Mexico City- *Journal of the American STD association* march 2001 Vol 28 issue 3.
4. *Kjaer SK, Svare El, Worm AM*- HPV infection in Danish female sex workers- decreased prevalence with age despite continuously high sexual activity- *Sexually Transmitted Disease Journal* september2000 vol 27 issue 8 pages 438-445.
5. *Stephen Goldstone, Joel M Palefsky, Anna R Giuliano*-Prevalence of and risk factors for HPV infection among HIV seronegative men who have sex with men- *J Infect dis*. 2011 volume 203 issue 1page 66-74.
6. *Gloria I. Sanchez, Bernhard Kleter, Tarik Gehit*- Clinical evaluation of PCR

   reverse hybridisation assay for detection and identification of HPV type 16 variants- *Journal of clinical virology*- 2011 june 2011 volume 51issue 3 pages 165- 169

7. *Nurena CR,Brown B,Galea JT* - HPV and genital warts among Peruvian men who have sex with men and transgender people: knowledge, attitude and treatment experience, *PLOS ONE* 2013 march volume 8 issue 3 e 58684.

8. *Rodriguez-Cerderia,Sanchez-Blanco E,Alba,A*- Evaluation of association between vaginal infections and High risk Human papilloma virus types in female sex workers in Spain ,ISRN Obstetrics and Gynecology 2012;2012:240190

9. *Fields Virology* 4th edition –Chapter 62 –*Papilloma virus* vol1 pages 2299-2340.

10. *Jawetz , Melnick and Adelberg's* Medical Microbiology 26th edition pages 644-647.

11. *Braun-Falco's Dermatology* volume 1,3rd edition pages 64-73.

12. *King Holmes, Evin M. Egelkrout and Denise A.Galloway - Sexually Transmitted Diseases* 4th edition, pages 463-507

13. IADVL Text book of Dermatology Volume 2, 3rd edition , pages 1914-1924.

14. *Vinod K.Sharma- Sexually Transmitted Diseases and HIV/AIDS* 2nd edition Chapter26, Anogenital warts pages 430-443.

15. *Bushan Kumar*- Sexually Transmitted Infections 2nd edition pages 360-373.

16. *Bailey and Scott's* Diagnostic Microbiology 13th edition pages 931- 944.

17. *Monica Cheesebrough* Second Edition Part 2 pages 90- 97 and 253- 260.

18. *Koneman's* color atlas and Textbook of Diagnostic Microbiology 6th edition pages- 566-623.

19. *NACO guidelines* for HIV testing- March 2007 Updated on September 2015.

20. *Connie R. Mahon* Textbook of Diagnostic Microbiology- page 364

21. *Andre' L P Abreu, Raquel P souza, Fabricia Gimenes*- A review of methods to detect *human papilloma virus* infection – *Virology* journal 2012, 9:262

22. *Pallavi Shukla ,Jamal, Masood ,V.K. Singh, JV Singh*-Predictors of Sexually Transmitted Infections among Female sex workers in a city of Northern India, *Indian J Community Med* 2015; 40:121-6.

23. *S Abarna Devi, TP Vetrichevvel, Gajanan A Pise, Devinder Mohan Thappa* Pattern of sexually transmitted infections in a tertiary care centre at Puducherry- *Indian J of Dermatol*. 2009 Oct- Dec 54(4):

24. *Dharma nand Bhatta*- HIV related sexual risk behavior among male to female Transgender people in Nepal, I*nternational Journal of Infectious Diseases* May 2014, vol .22:11-15.

25. *Susan Walsh* –Sexual statistics Review Session Feb 28, 2012.

26. R. Hemalatha, K Narendra Babu- Prevalence of and knowledge, attitude and practices towards HIV &Sexually Transmitted infections (STI's) among female sex workers in Andhra Pradesh, *Indian J Med Res* 134, Oct 2011, pp 470-475.

27. *Huy Ha, Jan M.H.Risser, Michael W.Ross*–Homosexuality– Related stigma and sexual risk behavior among Men who have sex with men in Hanoi ,Vietnam , *Archives of Sexual Behaviour* Feb 2015, volume 44,Issue 2, pp 349-356.

28. *Shilpee Choudry, V.G.Ramachandran*- Characterisation of patients with multiple sexually transmitted infections; A hospital based survey ,*IJSTD* 2010 June-Dec ;31 (2) 87-91.

29. *Tanmay Mahapatra, Subrata Biswas* – Burden and correlates of HIV among men Who have sex with men in West Bengal, India: Analysis of sentinel surveillance data, *PLoS one*.2015 ;10(5):e0127232.

30. Sexually transmitted infections (STIs) http://www.who.int/mediacentre/factsheets/fs110/en/

31. *Solomon MM, Smith MJ,del Rio C* – Low educational level :a risk factor for sexually Transmitted infections among Commercial sex workers in Quito, Ecuador, *Int J STD AIDS* 2008 Apr; 19(4) 264-7.

32. *Nimisha D. Shethwala , Summaiya Mulla ,Vikas K Desai* –Sexually Transmitted Infections and Reproductive tract infections in FSW , *IJPM* 2009,vol 52,Issue 2, pages 198-99.

33. *Vandana W.V,Saxena N.K*- A study on the prevalence of Gonorrhoea among High risk population in Hydrebad , AP, *Indian journal of public health research and Development* 2013 vol 4 Issue 1 pages 216-220.

34. HIV sentinel surveillance 2011-12 Technical brief –NACO

35. NACO, NACP Phase 4, 2011 – Strategic approach for targeted intervention among Transgenders and Hijras

36. Pisani E, Girault P, Gultom M –HIV ,Syphilis infection and sexual practices among Transgenders, male sex workers,and other Men having sex with men in Jakarta , Indonesia ,*Sex Transm Infect*.2004 Dec ;80 (6):536-40.

37. *Brahmam GN, Kodavalla V, Rajkumar H, Rachakulla HK, Kallam S, Myakala SP, Paranjape RS, Gupte MD, Ramakrishnan L, Kohli A, Ramesh BM*- Sexual practices, HIV and sexually transmitted infections among self identified Men who have sex with men in four high HIV prevalence states of India. IBBA Study Team- *AIDS*. 2008 Dec; 22 Suppl 5:S45-57.

38. *Uribe –Salas F,Del Rio –Chiriboga C,Conde-Glez CJ*- Prevalence, incidence,and determinants in female commercial sex workers in Mexico city, *Sex Transm Dis* 1996 Mar-Apr; 23(2):120-6.

39.. *Peng RR, Li HM, Chang H*- Prevalence and genotypic distribution of cervical HPV infection among female sex workers in Asia: a systematic literature review and meta- analysis- Sex health journal 2012 May; 9(2): 113-119

40. *Julia del Amo, Cristina Gonzalez*- Prevalence and risk factors of High risk Human papilloma virus in Female sex workers in Spain: Differences by geographical origin *Journal of Women's health*- December 2009, 18(12):2057-2064.

41. *Julia M.L.Botherton, David Smith*- HPV genotype prevalence in Australian women undergoing routine cervical screening *Journal of Clinical Virology* 60[2014] 250-256.

42. *Brandon Braun, Mariam Davtyan and Jeffrey D Klausner*- The role of Human papilloma virus in Human immunodeficiency virus acquisition in men who have sex with men: A review of the literature- *Viruses* 2012 December 4(12) 3851-3858.

43. *Mooji SH [1],Van der klis FR, Van der Sande MA*- Seroepidemiology of high risk HPV in HIV negative and HIV infected MSM: the H_2M study. *Cancer Epidemiol Biomarkers Prev*. 2013 oct; 22 (10) 1698-708.

44. *King EM[1] , Gilson R[2], Beddows S* - Human papilloma virus DNA in MSM-type specific prevalence, risk factors and implications in vaccination strategies- *Br J cancer* 2015 Apr.28;112(9):1585-93

45. *Maria a. Pando, Ivan C. Balan , Ruben Marone*- HIV and other sexually transmitted infections among men who have sex with Men recruited by RDS IN Buenos Aires , Argentina: High HIV and HPV infection –- *PLoS one* June 29, 2012.

46. *Nyitray, Alan G,Carvalho da silva, Roberto J,Mary MS* - The prevalence of genital HPV and factors associated with Oncogenic HPV among Men having sex with men and men having sex with women and men :The HIM study – *Sex Trans Dis* 2011 Oct 38(10) 932-940.

47. *Van Aar F,Mooji SH, Van der Sande* –Anal and penile High Risk Human papilloma virus prevalence in HIV negative and HIV infected MSM , *AIDS* 2013 Nov 28;27(18)2921-31.

48. *Alan G.Nyitray, Roberto j.Carvalho da Silva* - Age specific prevalence of risk factors for anal Human papilloma virus (HPV) among men who have

sex with women and men who have sex with men:The HPV in Men [HIM] study. *J Infec. Dis*.2011 Jan 1:203 (1) 49-57.

49. *Maria Sol dos Ramos Farias* - First report on sexually transmitted infection among Trans (male to female transvestites, transsexuals or transgenders)and MSWs in Argentina, high HIV,HPV,HBV, and Syphilis prevalence ,*Int J Infect.Dis* Sep 2011 vol 15(9) e 635-e.

50. *Brahman Prakash* –Reasons for sexual promiscuity in chemically dependent respondents and their awareness and acceptance of condom use, *Indian J.Prev.Soc.Med* . Vol. 34 No 1&2, 2003.

51. *Yan Luo,Chunyan Zhu, Rong Fu* – Risk factors for HIV & Syphilis infection among male sex workers who have sex with men: a cross sectional study in Hangzhou, China zoll , *BMJ* open 2015 ;5:e006791.

52. *Aritra Das ,Jian jun Li*-Factors associated with HIV & Syphilis co infection among MSM in seven Chinese cities, *Int J.STD & AIDS* 2015 Mar; 26(3):145-155.

53. *Lei Gao , Feng Zhou*- Anal HPV infection in HIV positive men who have sex with men from China , *PLoS ONE* 5 (12):E 15256.

54. *Alberts CJ [1], Van Rooijen MS, Prins M* - HIV is an important risk factor for Human papilloma virus types 16 and 18 seropositivity among sexually active men who have sex with men *Sex Trans Dis*.201`5 Mar:42(3) 129-34.

55. *Ishita Ghosh,Pramit Ghosh, Alok Chandra Bharti, Ranajit Mandal, Jaydip Biswas,Partha Basu*- Prevalence of Human Papillomavirus and Co-Existent Sexually Transmitted Infection among Female Sex Workers, Men having Sex

with Men and Injectable Drug abusers from Eastern India- *Asia Pacific J cancer Prev*, 13, 799- 802

.

PROFORMA

Sl No:

STD No:

Name:

Age:

Sex: M/ F/ TG

Marital status- Married (Y)/ unmarried (N)

Education: primary/ secondary/ college

Category: FSW/ MSM/ Promiscuous man/ Promiscuous woman/ Transgender

Mode of sex: Heterosexual/ Homosexual/ Bisexual

History of exposure: Extramarital (EMC)/ Premarital (PMC)

Last Marital contact:

Protected or Unprotected sex: Y/N

History of associated STI

History of Blood transfusion/ IV drug abuse/ Surgery

Examination findings: ulcer/ discharge/ wart

Types of swabs: high vaginal/ endocervical/ urethral/ rectal/ pharyngeal

Serology: RPR/ HIV

Sl. no	Std no.	sex	age	M Status Y/N	edu	Fsw	MSM / Csw	Pro. M	Pro. W	LMC	EMC	PMC	route	Prot sex	Blood Trans/ ivd	Asso. Std	Nature of specimen					RPR	HIV	hpv pcr
																	phary	rect	End cx	Hi Vag	ure			
1	320/15	M	19	N	10 std	NA	YES	NA	NA	NA	NA	YES	ANO REC	NO	NO	YES	NO	YES	NO	NO	NO	NR	NR	
2	334/15	M	30	Y	BA.,,	NA	NO	YES	NA	7 DAYS	YES	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
3	547/15	M	24	N	9 std	NA	YES	NA	NA	NA	NO	YES	ORO REC	YES	NO	NO	YES	NO	NO	NO	NO	NR	NR	
4	546/15	M	27	N	10 std	NA	YES	NA	NA	NA	NA	YES	ORO ANO REC	YES	NO	NO	YES	YES	NO	NO	NO	NR	REACTIVE	16
5	538/15	M	32	Y	8 std	NA	YES	NA	NA	4 YRS	YES	NO	ORO ANO REC & BISEX	YES	NO	NO	YES	YES	NO	NO	YES	NR	NR	
6	539/15	M	29	N	7 std	NA	YES	NA	NA	NA	NA	YES	ORO REC	YES	NO	NO	YES	NO	NO	NO	NO	NR	NR	
7	542/15	M	36	N	12 std	NA	YES	NA	NA	NA	NA	YES	ORO INS	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
8	550/15	M	32	Y	8 std	NA	YES	NA	NA	7 DAYS	YES	NO	ANO ORO REC & BISEX	YES	NO	YES	YES	YES	NO	NO	YES	NR	NR	18
9	549/15	M	48	Y	5 std	NA	YES	NA	NA	10 DAYS	YES	NO	ANO ORO REC & BISEX	YES	NO	NO	YES	YES	NO	NO	YES	NR	NR	
10	447/15	F	29	Y	8 std	YES	NA	NA	NA	2 DAYS	YES	NO	HETERO	NO	NO	YES	NO	NO	YES	YES	NO	N R	NR	
11	446/15	F	35	Y	6 std	YES	NA	NA	NA	1 DAY	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
12	443/15	F	33	Y	7 std	YES	NA	NA	NA	2 DAY	YES	NO	HETERO	NO	NO	YES	NO	NO	YES	YES	NO	NR	NR	
13	442/15	F	33	Y	8 std	YES	NA	NA	NA	10 DAYS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
14	418/15	F	35	Y	9 std	YES	NA	NA	NA	1 DAY	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
15	433/15	F	31	Y	7 std	YES	NA	NA	NA	2 WEEKS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
16	437/15	F	38	Y	10 std	YES	NA	NA	NA	1 WEEK	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
17	506/14	F	32	Y	5 std	NO	NA	NA	YES	5 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	REACTIVE	
18	426/15	F	30	Y	7 std	NA	NA	NA	YES	1 DAY	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
19	425/15	F	34	Y	4 std	NA	NA	NA	YES	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	

20	573/14	F	27	Y	6 std	NA	NA	NA	YES	1 DAY	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	16
21	427/15	F	26	Y	2 std	NA	NA	NA	YES	1 DAY	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
22	902/15	F	23	Y	10 std	NA	NA	NA	YES	1 DAY	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
23	932/15	F	36	Y	8 std	NA	NA	NA	YES	2 MONTHS	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
24	817/15	F	36	Y	10 std	NA	NA	NA	YES	2 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
25	971/15	F	30	Y	12 std	NA	NA	NA	YES	4 DAYS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
26	871/14	F	40	Y	8 std	NA	NA	NA	YES	3 YEARS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	REACTIVE	
27	1264/ 15	M	19	N	7 std	NA	YES	NA	NA	NA	NA	YES	ANO REC	NO	NO	YES	NO	YES	NO	NO	YES	NR	NR	
28	1451/ 15	M	25	N	8 std	NA	YES	NA	NA	NA	NA	YES	ORO ANO REC	YES	NO	NO	YES	YES	NO	NO	NO	NR	NR	
29	1452/ 15	M	19	N	12 std	NA	YES	NA	NA	NA	NA	YES	ORO ANO REC	YES	NO	YES	YES	YES	NO	NO	NO	NR	NR	
30	660/ 15	M	23	y	mphil	NA	NA	YES	NA	4 DAYS	NO	YES	HETERO	NO	NO	YES	NO	NO	NO	NO	YES	REACTIVE	NR	
31	688/ 15	M	42	Y	8 std	NA	NO	YES	NA	4 DAYS	YES	NO	HETERO	NO	NO	YES	NO	NO	NO	NO	YES	REACTIVE	NR	
32	1421/ 15	M	23	N	10 std	NA	NO	YES	NA	NA	NA	YES	HETERO	YES	NO	YES	NO	NO	NO	NO	YES	REACTIVE	NR	
33	1599/ 15	M	42	Y	12 std	NA	NO	YES	NA	2 MONTHS	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
34	617/ 15	M	42	Y	5 std	NA	NO	YES	NA	1 WEEK	NO	YES	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
35	1502/ 15	M	34	N	10 std	NA	NO	YES	NA	NA	NO	YES	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	REACTIVE	NR	
36	1503/ 15	M	39	Y	10 std	NA	NA	YES	NA	2 WEEKS	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
37	1380/ 14	M	20	N	5 std	NA	NA	YES	NA	2 DAYS	NO	YES	HETERO	NO	NO	YES	NO	NO	NO	NO	YES	NR	REACTIVE	
38	1202/ 15	M	30	Y	10 std	NA	NA	YES	NA	1 WEEK	YES	YES	HETERO	NO	NO	YES	NO	NO	NO	NO	YES	REACTIVE	NR	
39	1499/ 15	M	38	Y	iti	NA	NA	YES	NA	5 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
40	1495/ 15	M	46	Y	12 std	NA	YES	NO	NA	1 WEEK	YES	NO	ANO ORO REC & BISEX	YES	NO	NO	YES	YES	NO	NO	YES	NR	NR	

41	1494/ 15	M	25	N	10 std	NA	YES	NO	NA	NA	NA	YES	ORO REC	YES	NO	NO	YES	NO	NO	NO	NO	NR	NR	
42	1627/ 15	M	35	N	7 std	NA	YES	NO	NA	NA	NA	YES	ANO REC	YES	NO	NO	NO	YES	NO	NO	NO	NR	NR	
43	444/15	F	27	Y	10 std	YES	NA	NA	NO	2 DAYS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	16
44	441/15	F	39	Y	3 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
45	440/15	F	35	Y	2 std	YES	NA	NA	NO	5 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
46	439/15	F	40	Y	4 std	YES	NA	NA	NO	3 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
47	471/15	F	41	Y	5 std	YES	NA	NA	NO	8 DAYS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	REACTIV E	NR	
48	540/15	F	35	Y	3 std	NO	NA	NA	YES	4 DAYS	YES	YES	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	REACTIV E	NR	
49	432/15	F	40	Y	4 std	NO	NA	NA	YES	4 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
50	431/15	F	36	Y	8 std	NO	NA	NA	YES	2 MONTHS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	REACTIVE	
51	430/15	F	40	Y	5 std	NO	NA	NA	YES	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
52	524/15	F	25	Y	3 std	NO	NA	NA	YES	1 WEEK	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	REACTIV E	NR	
53	1213/ 15	F	36	Y	8 std	NO	NA	NA	YES	1 MONTH	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
54	424/15	F	26	Y	10 std	YES	NA	NA	NO	1 MONTH	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
55	1629/ 15	F	42	Y	7 std	YES	NA	NA	NO	10 DAYS	YES	NO	HETERO	NO	NO	YES	NO	NO	YES	YES	NO	NR	NR	
56	1214/ 15	F	35	Y	4 std	YES	NA	NA	NO	20 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
57	1216/ 15	F	34	Y	8 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
58	1219/ 15	F	24	Y	5 std	YES	NA	NA	NO	1 MONTH	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	18
59	1220/ 15	F	26	Y	9 std	YES	NA	NA	NO	3 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
60	1221/ 15	F	29	N	6 std	YES	NA	NA	NO	NA	NA	YES	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
61	1217/ 15	F	30	Y	2 std	YES	NA	NA	NO	1 MONTH	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	

62	1222/ 15	F	35	Y	2 std	YES	NA	NA	NO	2 WEEKS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
63	1218/ 15	F	30	Y	3 std	YES	NA	NA	NO	1 MONTH	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
64	1583/ 15	M	25	N	10 std	NA	YES	NO	NA	NA	NA	YES	ANO ORO REC INS	NO	NO	YES	YES	YES	NO	NO	YES	NR	NR	
65	1556/ 15	M	44	Y	7 std	NA	YES	NO	NA	2 WEEKS	YES	NO	ORO INS & BISEX	YES	NO	NO	YES	NO	NO	NO	YES	NR	NR	
66	1661/ 15	M	21	N	BE	NA	YES	NO	NA	NA	NA	YES	ANO ORO REC	NO	NO	NO	YES	YES	NO	NO	NO	NR	NR	
67	367/15	M	30	N	2 std	NA	YES	NO	NA	NA	NA	YES	ORO ANO REC INS	NO	NO	NO	YES	YES	NO	NO	YES	NR	NR	
68	391/ 15	M	35	N	10 std	NA	NO	YES	NA	NA	NA	YES	HETERO	YES	NO	YES	NO	NO	NO	NO	YES	REACTIV E	NR	
69	190/ 15	M	38	N	8 std	NA	NO	YES	NA	NA	NA	YES	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	REACTIV E	NR	
70	1559/ 15	M	28	Y	Deg	NA	NO	YES	NA	3 MONTHS	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
71	1557/ 15	M	37	N	5 std	NA	NO	YES	NA	NA	NA	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
72	1555/ 15	F	31	N	4 std	YES	NO	NO	NO	NA	NA	YES	HETERO	NO	NO	YES	NO	NO	NO	NO	YES	NR	NR	
73	1139/ 14	M	43	Y	4 std	NA	NO	YES	NA	3 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	REACTIVE	
74	1550/ 15	M	39	Y	12 std	NA	NO	YES	NA	4 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
75	1551/ 15	M	48	Y	3 std	NA	NO	YES	NA	3 WEEKS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
76	1539/ 15	M	28	N	8 std	NA	NO	YES	NA	NA	NA	YES	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
77	1437/ 14	M	31	N	ITI	NA	NO	YES	NA	NA	NA	YES	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	REACTIVE	
78	1105/ 14	M	23	Y	6 std	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	16
79	1588/ 15	M	28	Y	12 std	NA	NO	YES	NA	2 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
80	1671/ 15	M	40	Y	7 std	NA	NO	YES	NA	1 DAY	YES	NO	HETERO	NO	IVD	NO	NO	NO	NO	NO	YES	NR	NR	
81	1670/ 15	M	40	Y	11 std	NA	NO	YES	NA	10 DAYS	YES	NO	HETERO	NO	IVD	NO	NO	NO	NO	NO	YES	NR	NR	
82	52/15	M	28	Y	BA	NA	NO	YES	NA	3 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	

83	1247/ 15	F	21	Y	8 std	YES	NA	NA	NO	2 MONTHS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
84	1246/ 15	F	28	Y	10 std	YES	NA	NA	NO	3 DAYS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
85	263/15	F	35	Y	9 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	MO	NR	NR	
86	357/15	F	31	Y	6 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
87	299/15	F	31	Y	10 std	YES	NA	NA	NO	5 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
88	298/15	F	31	Y	10 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
89	283/15	F	24	Y	5 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
90	282/15	F	25	Y	3 std	YES	NA	NA	NO	2 WEEKS	YES	NO	HETERO	NO	NO	YES	NO	NO	YES	YES	NO	NR	NR	
91	1158/ 15	F	33	Y	10 std	NO	NA	NA	YES	1 DAY	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
92	308/15	F	26	Y	7 std	NO	NA	NA	YES	1 WEEK	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
93	35/15	F	30	Y	3 std	NO	NA	NA	YES	4 DAYS	NO	YES	HETERO	NO	NO	YES	NO	NO	YES	YES	NO	NR	NR	
94	423/15	F	40	Y	10 std	NO	NA	NA	YES	10 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
95	281/15	F	30	Y	2 std	YES	NA	NA	NO	2 WEEKS	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
96	422/15	F	38	Y	10 std	YES	NA	NA	NO	1 MONTH	YES	NO	HETERO/ ORAL	NO	NO	NO	YES	NO	YES	YES	NO	NR	NR	
97	421/15	F	38	Y	5 std	YES	NA	NA	NO	1 MONTH	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
98	303/15	F	27	Y	6 std	YES	NA	NA	NO	1.5 MONTH	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
99	304/15	F	37	Y	7 std	YES	NA	NA	NO	4 MONTHS	YES	NO	HETREO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
100	305/15	F	36	Y	4 std	YES	NA	NA	NO	4 MONTHS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
101	306/15	F	30	Y	2 std	YES	NA	NA	NO	3 MONTHS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
102	284/15	F	24	Y	4 std	YES	NA	NA	NO	2 MONTHS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
103	285/15	F	35	Y	3 std	YES	NA	NA	NO	2 WEEKS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	

104	286/15	F	40	Y	7 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
105	287/15	F	40	Y	5 std	YES	NA	NA	NO	15 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
106	288/15	F	27	Y	7 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	YES	NO	NO	NO	YES	YES	NO	NR	NR	
107	289/15	F	35	Y	3 std	YES	NA	NA	NO	10 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
108	290/15	F	31	Y	9 std	YES	NA	NA	NO	4 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
109	291/15	F	35	Y	7 std	YES	NA	NA	NO	1 MONTH	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
110	292/15	F	25	N	10 std	YES	NA	NA	NO	NA	NA	YES	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
111	293/15	F	27	Y	4 std	YES	NA	NA	NO	3 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	16 18
112	294/15	F	30	Y	5 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
113	295/15	F	35	Y	6 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
114	1687/ 15	M	28	Y	11 std	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	REACTIV E	NR	
115	1259/ 15	F	20	Y	10 std	NO	NA	NA	YES	1 WEEK	YES	NO	HETERO	NO	NO	YES	NO	NO	YES	YES	NO	REACTIV E	NR	
116	1694/ 15	M	37	Y	11 std	NA	NO	YES	NA	2 DAYS	YES	NO	HETERO	YES	YES	NO	NO	NO	NO	NO	YES	NR	NR	
117	1578/ 15	M	27	N	5 std	NA	NA	YES	NA	NA	NO	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
118	1838/ 15	M	34	Y	7 std	NA	YES	NO	NA	4 DAYS	YES	NO	ANO INS & BISEX	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
119	1665/ 15	M	34	Y	9 std	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
120	1854/ 15	M	45	N	8 std	NA	YES	NO	NA	NA	NA	YES	ANO REC & BISEX	YES	NO	NO	NO	YES	NO	NO	YES	NR	NR	
121	290/15	M	24	N	8 std	NA	YES	NO	NA	NA	NA	YES	ORO ANO – REC & INS	NO	NO	YES	YES	YES	NO	NO	YES	NR	NR	
122	1810/ 15	M	27	N	mba	NA	NO	YES	NA	NA	NA	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
123	1748/ 15	M	44	Y	7 std	NA	NO	YES	NA	5 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
124	1849/ 15	M	32	Y	12 std	NA	NO	YES	NA	10 DAYS	YES	NO	HETREO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	

125	560/15	M	38	Y	10 std	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
126	1388/ 15	F	18	N	9 std	YES	NA	NA	NA	NA	NA	YES	HETERO	NO	NO	YES	NO	NO	YES	YES	NO	NR	NR	16
127	1853/ 15	F	40	Y	iti	YES	NO	NO	NA	10 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
128	1850/ 15	F	28	N	7 std	YES	NO	NO	NA	NA	NA	YES	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
129	1786/ 15	M	28	Y	5 std	NA	NO	YES	NA	10 DAYS	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
130	1861/ 15	M	30	N	6 std	NA	YES	NO	NA	NA	NA	YES	ANO REC & BISEX	YES	NO	YES	NO	YES	NO	NO	YES	NR	NR	
131	1355/ 15	M	44	Y	8 std	NA	NO	YES	NA	1 MONTH	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	REACTIV E	NR	
132	1858/ 15	M	24	N	BE	NA	NO	YES	NA	NA	NA	YES	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
133	1716/ 15	M	39	Y	10 std	NA	NO	YES	NA	4 WEEKS	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
134	1860/ 15	M	24	N	B com	NA	NO	YES	NA	NA	NO	YES	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
135	1804/ 15	M	34	Y	9 std	NA	NO	YES	NA	NO	NO	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	REACTIVE	
136	549/ 15	F	47	Y	8 std	NO	NA	NA	YES	NO	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
137	1397/ 15	F	28	Y	4 std	YES	NA	NA	NO	1DAY	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
138	1398/ 15	F	34	y	8 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
139	1399/ 15	F	43	Y	7 std	YES	NA	NA	NO	10 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
140	1400/ 15	F	42	Y	6 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
141	1401/ 15	F	36	Y	5 std	YES	NA	NA	NO	2 WEEKS	YES	NO	HETERO	NO	NO	YES	NO	NO	YES	YES	NO	NR	NR	
142	1402/ 15	F	35	Y	4 std	YES	NA	NA	NO	1 WEEK	YES	NO	HETERO	YES	NO	NO	NO	NO	YES	YES	NO	NR	NR	
143	74/14	F	26	Y	2 std	NO	NA	NA	YES	15 DAYS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	REACTIV E	REACTIVE	
144	1043/ 15	F	27	Y	6 std	NO	NA	NA	YES	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	YES	NR	NR	
145	1447/ 14	M	25	N	Deg	NA	NO	YES	NA	NA	NO	YES	HETERO	YES	NO	YES	NO	NO	NO	NO	YES	REACTIV E	REACTIVE	

146	1905/ 15	M	24	Y	Dip	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
147	1324/ 14	M	35	N	6 std	NA	NO	YES	NA	NA	NO	YES	HETERO	NO	NO	YES	NO	NO	NO	NO	YES	REACTIVE	REACTIVE	
148	1918/ 15	M	34	N	10 std	NA	NO	YES	NA	NA	NA	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
149	1919/ 20	M	34	N	10 std	NA	NO	YES	NA	NA	NA	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
150	1866/ 15	M	21	N	12 std	NA	NO	YES	NA	NA	NA	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
151	1920/ 15	M	30	N	10 std	NA	YES	NO	NA	NA	NA	YES	ANO ORO INS REC	NO	NO	NO	YES	YES	NO	NO	YES	NR	NR	
152	1567/ 15	M	29	N	BA	NA	YES	NO	NA	NA	NA	YES	ORO INS	NO	NO	YES	YES	YES	NO	NO	YES	REACTIVE	NR	
153	1923/ 15	M	26	Y	8 std	NA	NO	YES	NA	1 MONTH	NO	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
154	1924/ 15	M	42	Y	10 std	NA	NO	YES	NA	20 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
155	1926/ 15	M	30	Y	12 std	NA	NO	YES	NA	6 WEEKS	YES	NO	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
156	1925/ 15	M	36	N	10 std	NA	YES	NO	NA	NA	NA	YES	ANO INS	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
157	1935/ 15	M	30	N	8 std	NA	YES	NO	NA	NA	NA	YES	ANO REC	YES	NO	NO	NO	YES	NO	NO	NO	NR	NR	
158	1929/ 15	M	36	Y	10 std	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
159	1934/ 15	M	25	N	Dip	NA	NO	YES	NA	NA	NO	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
160	1933/ 15	M	47	Y	4 std	NA	NO	YES	NA	5 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
161	1936/ 15	M	23	N	6 std	NA	NO	YES	NA	NA	NO	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
162	1958/ 15	M	40	Y	8 std	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
163	1963/ 15	M	34	Y	12 std	NA	NO	YES	NA	3 WEEKS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
164	1904/ 15	M	35	N	10 Std	NA	NO	YES	NA	NA	NO	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
165	1957/ 15	M	43	Y	10 std	NA	NO	YES	NA	4 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
166	1965/ 15	M	35	Y	7 std	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	REACTIVE	

167	1956/ 15	M	47	Y	6 std	NA	NO	YES	NA	4 WEEKS	YES	NO	HETERO	NO	NO	YES	NO	NO	NO	NO	YES	NR	NR	
168	1953/ 15	M	46	Y	Iti	NA	NO	YES	NA	5 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
169	184/ 15	M	38	Y	10 std	NA	NO	YES	NA	4 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
170	1439/ 15	F	49	Y	6 std	YES	NA	NO	NO	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NR	
171	1441/ 15	F	26	Y	4 std	YES	NA	NO	NO	5 DAYS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NR	
172	1442/ 15	F	26	Y	8 std	YES	NA	NO	NO	2 DAYS	YES	NO	HETERO	NO	NO	YES	NO	NO	YES	YES	NO	NR	NR	
173	1443/ 15	F	41	Y	5 std	YES	NA	NO	NO	3 WEEKS	YES	NO	HETERO	NO	NO	NO	NO	NO	YES	YES	NO	NR	NEG	
174	1440/ 15	F	30	Y	7 std	YES	NA	NO	NO	2 WEEKS	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NEG	
175	1276/ 15	F	30	Y	10 std	YES	NA	NO	NO	1 DAY	YES	NO	HETERO	YES	NO	YES	NO	NO	YES	YES	NO	NR	NEG	
176	1818/ 15	M	32	N	5 std	NA	YES	NO	NA	NA	NA	YES	ORO INS	NO	NO	NO	NO	NO	NO	NO	YES	NR	NEG	
177	1757/ 15	M	26	N	7 std	NA	YES	NO	NA	NA	NA	YES	ANO REC & INS	YES	NO	YES	NO	YES	NO	NO	YES	NR	NEG	
178	1758/ 15	M	22	N	10 std	NA	YES	NO	NA	NA	NA	YES	ORO INS	YES	NO	NO	NO	NO	NO	NO	YES	NR	NEG	
179	1676/ 15	M	32	Y	8 std	NA	YES	NO	NA	1 WEEK	YES	NO	ORO REC & BISEX	NO	NO	NO	YES	NO	NO	NO	YES	NR	NR	
180	1863/ 15	TG	28	N	8 std	CSW	NA	NA	N0	NA	NA	YES	ORO ANO REC	YES	NO	NO	YES	YES	NO	NO	NO	NR	NR	
181	14/ 15	TG	25	N	3 std	CSW	NA	NA	NO	NA	NA	YES	ORO ANO REC	YES	NO	NO	YES	YES	NO	NO	NO	NR	NR	
182	4/ 15	TG	30	N	5 std	CSW	NA	NA	NO	NA	NA	YES	ORO ANO REC	YES	NO	NO	YES	YES	NO	NO	NO	NR	NR	
183	5/15	TG	20	N	7 std	CSW	NA	NA	NO	NA	NA	YES	ORO ANO REC	YES	NO	NO	YES	YES	NO	NO	NO	REACTIV E	NR	
184	6/ 15	TG	24	N	9 std	CSW	NA	NA	NO	NA	NA	YES	ORO ANO REC	YES	NO	NO	YES	YES	NO	NO	NO	REACTIV E	REACTIVE	
185	932/ 14	TG	31	N	6 std	CSW	NA	NA	NO	NA	NA	YES	ORO ANO REC	NO	NO	NO	YES	YES	NO	NO	NO	NR	REACTIVE	
186	8/ 15	TG	40	Y	3 std	CSW	NA	NA	NO	NA	NA	YES	ORO ANO REC	YES	NO	NO	YES	YES	NO	NO	NO	NR	NR	
187	1832/ 15	M	45	Y	5 std	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	

188	1821/ 15	M	36	Y	4 std	NA	NO	YES	NA	3 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
189	1946/ 15	M	30	Y	2 std	NA	NO	YES	NA	2 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
190	1945/ 15	M	35	Y	8 std	NA	NO	YES	NA	2 WEEKS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
191	1809/ 15	M	46	Y	3 std	NA	NO	YES	NA	4 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
192	1792/ 15	M	36	Y	6 std	NA	NO	YES	NA	2 DAYS	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
193	1790/ 15	M	29	N	4 std	NA	NO	YES	NA	NA	NO	YES	HETERO	YES	NO	NO	NO	NO	NO	NO	YES	NR	NR	
194	1707/ 15	M	44	N	3 std	NA	NO	YES	NA	NA	NO	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
195	1699/ 15	M	24	N	5 std	NA	NO	YES	NA	NA	NO	YES	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
196	1696/ 15	M	34	Y	2 std	NA	NO	YES	NA	1 WEEK	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	NR	NR	
197	1670/ 15	M	40	Y	10 std	NA	NO	YES	NA	4 DAYS	YES	NO	HETERO	NO	IVD	NO	NO	NO	NO	NO	YES	NR	NR	
198	1671/ 15	M	40	Y	8 std	NA	NO	YES	NA	2 DAYS	YES	NO	HETERO	NO	IVD	NO	NO	NO	NO	NO	YES	NR	NR	
199	209/ 15	M	41	Y	4 std	NA	NO	YES	NA	2 DAYS	YES	NO	HETERO	NO	NO	YES	NO	NO	NO	NO	YES	NR	NR	
200	1682/ 15	M	30	Y	3 std	NA	NO	YES	NA	1 DAY	YES	NO	HETERO	NO	NO	NO	NO	NO	NO	NO	YES	REACTIV E	NR	

Key to Master Chart

Sl. No- Serial number

FSW- Female sex worker

MSM- Men having sex with men

Prom. M- Promiscuous men

Prom. W- Promiscuous women

TG- Transgender

Hetero- Heterosexual

Ano- anal

Oro- Oral

Rec- Receptive

Ins- Insertive

Bisex-Bisexual

Prot. Sex- Protected sex

EMC- Extra marital contact

PMC- Premarital contact

LMC- Last Marital contact

Asso. Disease- Associated Sexually Transmitted Illnesses

IVD- IV drug abuse

Blood trans- Transfusion

Endo cer- Endocervical

URe- Urethral

Rect- Rectal

Hi.VAG- High vaginal

Phary- Pharyngeal

RPR- Rapid Plasma Reagin

HIV- Human Immunodeficiency virus

HPV PCR- Human Papilloma Virus Polymerase Chain Reaction

NA-Not applicable

NR- Non Reactive